SHOUT
SHARE
IT

LEATHER DRESSING
JAN- 1967

P
11
Botany
9
89612
Smith

AMERICAN

JOURNAL OF BOTANY

OFFICIAL PUBLICATION OF THE
BOTANICAL SOCIETY OF AMERICA

EDITORIAL COMMITTEE

C. E. ALLEN, Editor-in-Chief
University of Wisconsin

WILLIAM CROCKER,
University of Chicago

L. R. JONES,
University of Wisconsin

C. STUART GAGER, Business Manager
Brooklyn Botanic Garden

ARTHUR H. GRAVES,
Brooklyn Botanic Garden

R. A. HARPER,
Columbia University

JACOB R. SCHRAMM,
Cornell University

C. L. SHEAR, *Bureau of Plant Industry*
(Representing American Phytopathological Society)

3

VOLUME VIII—1921

WITH TWENTY-SIX PLATES AND NINETY-FIVE TEXT FIGURES

PUBLISHED
IN COOPERATION WITH THE BOTANICAL SOCIETY OF AMERICA
BY THE
BROOKLYN BOTANIC GARDEN
AT 41 NORTH QUEEN STREET, LANCASTER, PA.

Smithsonian Institution
FEB 18 1922
283599
National Museum

PRESS OF
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

TABLE OF CONTENTS, VOLUME VIII, 1921

No. 1, January

PAGE
The fixation of free nitrogen by green plants (with one text figure and Plate I) . . . . Frank B. Wann 1
Variations in the osmotic concentrations of the guard cells during the opening and closing of stomata (with seven text figures) R. G. Wiggans 30
The Linnaean concept of pearl millet . . . . Agnes Chase 41
The effect of cloudiness on the oxygen content of water and its significance in cranberry culture (with three text figures) H. F. Bergman 50

No. 2, February

Influence of temperature on the relations between nutrient salt proportions and the early growth of wheat . . . . W. F. Gericke 59
The vascular anatomy of dimerous and trimerous seedlings of Phaseolus vulgaris (with twenty-three text figures) J. Arthur Harris, Edmund W. Sinnott, John Y. Pennypacker, and G. B. Durham 63
Aspergillus flavus, A. oryzae, and associated species (with one text figure) . . . . Charles Thom and Margaret B. Church 103

No. 3, March

A study of Rhus diversiloba with special reference to its toxicity (with two text figures and Plate II) . . . . James B. McNair 127
The effect of salt proportions and concentration on the growth of Aspergillus niger (with six text figures) . . . . C. M. Haenseler 147
Suggestions with respect to the measurement of osmotic pressure (with one text figure) . . . . L. Knudson and S. Ginsburg 164
Thick-walled root hairs of Gleditsia and related genera (with three text figures) . . . . W. B. McDougall 171
A simple method for growing plants (with one text figure) J. M. Brannon 176

No. 4, April

The morphology and anatomy of Rhus diversiloba (with Plates III and IV) . . . . James B. McNair 179

Distribution of the Malvaceae in southern and western Texas (with one text figure) . . . . HERBERT C. HANSON 192
Note on the histology of grain roots (with four text figures) GRACE A. DUNN 207
North American Pipers of the section Ottonia (with Plates V–VIII) WILLIAM TRELEASE 212
Monocarpy and pseudomonocarpy in the cycadeoids (with one text figure and Plates IX–XII) . . . . G. R. WIELAND 218

No. 5, MAY

Isoachlya, a new genus of the Saprolegniaceae (with Plates XIII and XIV) . . . . C. H. KAUFFMAN 231
The transmission of Rhus poison from plant to person JAMES B. MCNAIR 238
The type concept in systematic botany . . . . A. S. HITCHCOCK 251
The relation of certain nutritive elements to the composition of the oat plant (with two text figures) . . . . JAMES GEERE DICKSON 256

No. 6, JUNE

Specialization and fundamentals in botany . . JOSEPH CHARLES ARTHUR 275
Certain aspects of the problem of physiological correlation C. M. CHILD 286
Water deficit and the action of vitamines, amino-compounds, and salts on hydration . . . . D. T. MACDOUGAL 296
The eusporangiate ferns and the stelar theory (with seven text figures) D. H. CAMPBELL 303
The relation of plant pathology to human welfare . . . . . F. L. STEVENS 315

No. 7, JULY

The relation of crop-plant botany to human welfare CARLETON R. BALL 323
Correlations between anatomical characters in the seedling of Phaseolus vulgaris (with eight text figures) J. ARTHUR HARRIS, EDMUND W. SINNOTT, JOHN Y. PENNYPACKER, and G. B. DURHAM 339
A quantitative study of the effect of anions on the permeability of plant cells II (with one text figure) . . . . ORAN L. RABER 366
The mechanism of root pressure and its relation to sap flow JAMES BERTRAM OVERTON 369

No. 8, OCTOBER

The vascular anatomy of hemitrimerous seedlings of Phaseolus vulgaris J. ARTHUR HARRIS, EDMUND W. SINNOTT, JOHN Y. PENNYPACKER, and G. B. DURHAM 375

The effect upon permeability of polyvalent cations in combination with polyvalent anions (with one text figure) . . . . ORAN L. RABER 382
The floral anatomy of the Urticales (with Plates XV–XXII). ALBERT REIFF BECHTEL 386
Genetic evidence of aberrant chromosome behavior in maize endosperm (with one text figure) . . . . R. A. EMERSON 411

No. 9, NOVEMBER

The interrelationship of the number of the two types of vascular bundles in the transition zone of the axis of Phaseolus vulgaris (with two text figures)
J. ARTHUR HARRIS, EDMUND W. SINNOTT, JOHN Y. PENNYPACKER, and G. B. DURHAM 425
Area of vein-islets in leaves of certain plants as an age determinant (with Plate XXIII) . . . . M. R. ENSIGN 433
Unusual rusts on Nyssa and Urticastrum (with six text figures) E. B. MAINS 442
Miscellaneous studies on the crown rust of oats (with Plate XXIV) G. R. HOERNER 452
Comparative studies on respiration XVIII. Respiration and antagonism in Elodea (with two text figures) . . . . C. J. LYON 458
The effect upon permeability of (I) the same substance as cation and anion, and (II) changing the valency of the same ion (with two text figures) . . . . ORAN L. RABER 464

No. 10, DECEMBER

Pollen and pollen enzymes . . . . JULIA BAYLES PATON 471
The embryogeny of Cyrtanthus parviflorus Baker (with Plates XXV and XXVI) . . . . WM. RANDOLPH TAYLOR 502
Studies on plant cancers III. The nature of the soil as a determining factor in the health of the beet, Beta vulgaris, and its relation to the size and weight of the crown gall produced by inoculation with Bacterium tumefaciens (with nine text figures) MICHAEL LEVINE 507
Index to Volume VIII 526

(Dates of publication: No. 1, Mar. 9; No. 2, Mar. 19; No. 3, Apr. 3; No. 4, Apr. 30; No. 5, May 24: No. 6, June 30; No. 7, Aug. 31; No. 8, Nov. 14; No. 9, Dec. 19; No. 10, Feb. 15, (1922.)

ERRATA, VOLUME VIII

Page 31, table I: *Eryngium campestra* should be *Eryngium campestre.*

Page 145, 7th line should read:

26. **Hooker, W. J.** Flora boreali-americana **1**: 126, 127. London, 1833.

8th and 9th lines should read:

27. **Hooker, W. J., and Arnott, G. A. W.** The botany of Captain Beechey's voyage, part 3, p. 137. London, 1841.

28th line should read:

Lyon, W. S. The flora of our southwestern archipelago II. Bot. Gaz. **11** 330–336. 1886.

Page 146, 9th line should read:

London, 1838.

In line 14:

New Yor should be *New York.*

Page 195, line 26: *Malva parvifolia* should be *Malva parviflora.*

Page 231, 1st line of text

After *Kauffman,* add *and Coker*

Page 274, 3d line should read:

Wolff, E. 1871. Aschen Analysen **1**: 1–149; **2**: 1–170.

Page 451, 18th line: *Urticastri* should be *Dicentrae*

Vol. VIII JANUARY, 1921 No. 1

AMERICAN
JOURNAL OF BOTANY

OFFICIAL PUBLICATION OF THE
BOTANICAL SOCIETY OF AMERICA

Smithsonian Institution
MAR 15 1921
National Museum

CONTENTS

The fixation of free nitrogen by green plants . . . . FRANK B. WANN 1

Variations in the osmotic concentrations of the guard cells during the opening and closing of stomata . . . . R. G. WIGGINS 30

The Linnean concept of pearl millet . . . . AGNES CHASE 41

The effect of cloudiness on the oxygen content of water and its significance in cranberry culture . . . . H. F. BERGMAN 50

PUBLISHED
IN COOPERATION WITH THE BOTANICAL SOCIETY OF AMERICA
AT 41 NORTH QUEEN STREET, LANCASTER, PA.
BY THE
BROOKLYN BOTANIC GARDEN
BROOKLYN, N. Y.

Entered as second-class matter February 21, 1914, at the post office at Lancaster, Pennsylvania, under the act of March 3, 1879

AMERICAN JOURNAL OF BOTANY

DEVOTED TO ALL BRANCHES OF BOTANICAL SCIENCE

ESTABLISHED 1914

EDITED BY A COMMITTEE OF THE
BOTANICAL SOCIETY OF AMERICA

EDITORIAL COMMITTEE

C. E. ALLEN, Editor-in-Chief,
University of Wisconsin

WILLIAM CROCKER,
University of Chicago

L. R. JONES,
University of Wisconsin

C. STUART GAGER, Business Manager
Brooklyn Botanic Garden

ORLAND E. WHITE,
Brooklyn Botanic Garden

R. A. HARPER,
Columbia University

JACOB R. SCHRAMM,
Cornell University

C. L. SHEAR, *Bureau of Plant Industry*
(Representing American Phytopathological Society)

The Journal is published monthly, except during August and September. Subscription price, $6.00 a year. Single copies 75 cents. Back numbers, 75 cents each; $6.00 a volume, postage extra. Postage will be charged to all foreign countries, except Mexico, Cuba, Porto Rico, Panama Canal Zone, Republic of Panama, Hawaii, Philippine Islands, Guam, Samoan Islands, and Shanghai. Postage to Canada, 20 cents a volume on annual subscriptions; to all other countries in the Postal Union, 40 cents a volume on annual subscriptions.

The pages of the Journal are open to members of the Botanical Society of America, or to candidates approved for membership.

Manuscript offered for publication should be typewritten, and should in all cases be submitted to the Editor-in-Chief.

Papers are limited to 20 pages in length, exclusive of plates, except that additional pages may be arranged for at cost rate to authors (approximately $3.00 a page).

Proofs should be corrected immediately on receipt, and returned to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y.

Illustrations, other than zinc etchings, and tabular matter should, in general, be restricted to approximately ten percent of the number of pages of text. Authors may be charged at cost rates for illustrations and tabular matter in excess of that amount (approximately $6.40 a page for tabular matter; 15 cents per square inch for half-tones).

Separates should be ordered when proof is returned. Fifty copies without cover will be supplied free; cover and additional copies at cost.

Remittances should be made payable to American Journal of Botany. Ten cents must be added to all checks not drawn on New York City banks.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

Correspondence concerning editorial matters should be addressed to Prof. C. E. Allen, University of Wisconsin, Madison, Wisconsin.

Business correspondence, including notice of change of address, and directions concerning reprints, should be addressed to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y., or 41 North Queen Street, Lancaster, Pa.

AMERICAN
JOURNAL OF BOTANY

VOL. VIII JANUARY, 1921 NO. 1

THE FIXATION OF FREE NITROGEN BY GREEN PLANTS

FRANK B. WANN

(Received for publication July 3, 1920)

The ability of chlorophyll-bearing plants to utilize the uncombined nitrogen of the atmosphere has been repeatedly investigated during the last three or four decades, and the results of numerous observations and experiments, covering a wide range of species, have been quite conflicting. In some of the earlier experiments with higher plants in pot cultures the beneficial effect of a surface layer of algae was often observed, and the ability to increase the nitrogen content of the soil by free nitrogen fixation was ascribed to members of both the blue-green (Cyanophyceae) and grass-green (Chlorophyceae) algae. Similar increases in soil-nitrogen content were observed when higher plants were excluded from the cultures, and, though bacteria were known to be present, the fixation was generally ascribed to the chlorophyll-containing forms. More recently, pure cultures of members of the Chlorophyceae have been used but the results in these cases have been almost uniformly negative. This fact, together with the discovery of widely distributed soil bacteria of the Azotobacter and Clostridium types, the ability of which to fix free nitrogen can not be questioned, has led to the belief that in impure cultures fixation is due not to the activities of the green plants but to the bacteria present in the soil. Thus it has come to be very generally accepted that members of the Chlorophyceae, as well as the higher plants, are not able to use free nitrogen.

However, the number of species which have been investigated in pure culture is small, and the culture methods employed have not always been those which are most favorable for the best growth of these organisms. Accordingly the experiments reported here were undertaken for the purpose of extending the observations over a larger number of species, grown on a variety of mineral nutrient solutions under culture conditions which would insure a rapid and vigorous growth.

LITERATURE

A complete, and in some cases detailed, review of the literature bearing on the relation of the grass-green algae (Chlorophyceae) to free nitrogen is available in a paper by Schramm (1914 *a*), so that a repetition of the account is unnecessary here. Nothing of importance relating to this subject has

[The *Journal* for December (7: 409–468) was issued January 12, 1921.]

appeared, so far as the author is aware, since the above-cited paper. As has already been indicated, the results of experiments with pure cultures have been pretty generally negative as regards the ability of these forms to increase the nitrogen content of the culture. In the light of results presented here, some of the previous experimental work will be considered in the general discussion to follow.

METHODS

Seven species of Chlorophyceae were isolated and used in pure culture. In making the isolations the plate culture method, as described by Schramm (1914 *b*), was employed. With the exception of one species, *Protococcus* sp., all isolations were made from growths occurring on soil; the material for the Protococcus culture was secured from the bark of an elm tree.

The absolute identity of all the species has not as yet been determined. Cultures have been submitted to several authorities on the group, but for some of the forms the determinations received have been somewhat at variance. For that reason no attempt has been made to apply specific names to all the organisms. Moreover, as will be apparent later, the absolute identity of the forms, though highly desirable, does not become of paramount importance because of the very similar way in which all the species seem to react. Unless otherwise indicated, therefore, the different species will be referred to by number and genus, or by number only, and as soon as more satisfactory determinations can be made a list will be published, if possible, in this journal. The forms used in the experiments include the following:

Species number 1. *Chlorella vulgaris* Beyr. There seems to be no doubt about the identity of this species.

Species number 2. *Stichococcus* sp.

Species number 3. *Protosiphon botryoides* (Kg.) Klebs.

Species number 5. *Chlorella* sp. A small form with cup-chaped chromatophore.

Species number 6. *Scenedesmus* sp.

Species number 7. *Protococcus* sp.

Species number 11. *Chlorella* sp. A large form with clathrate chromatophore.

All these species have been carried along on mineral nutrient agar for two or three years; they have been repeatedly transferred to media containing glucose or sucrose, and have frequently been examined microscopically. They are known to be free from bacteria and are pure cultures in the strict sense.

Culture Media.—In the experiments Kjeldahl flasks of Pyrex glass and of 500 cc. capacity were used as culture flasks, because of the obvious advantage of analyzing checks and cultures without transferring the material to a digestion flask. Approximately 150 grams of mineral nutrient agar were supplied as a medium for each culture. In spite of the difficulties involved in its analysis, a solid medium was chosen because of the very long-continued, vigorous growth produced on it. So far as has been observed, solution cultures, at least when unaerated, do not give a very extended or abundant development of these organisms. Since many previous experiments have also shown that these forms do not grow in pure culture in the complete absence of combined nitrogen, no attempt was made to include such cultures in the experiments.

Two experiments were performed, the first in the winter of 1917–18 and the second in the summer of 1919. In both cases the following mineral nutrient solution was employed as a standard for the preparation of the media:

NH_4NO_3	0.5 gram
$MgSO_4$	0.2 gram
K_2HPO_4	0.2 gram
$CaCl_2$	0.1 gram
$FeSO_4$	trace
Distilled water	1000 cc.

With the nitrogen content of this solution as a basis, the NH_4NO_3 was replaced in the various series of 1917–18 by glycocoll, asparagine, $(NH_4)_2SO_4$, and $Ca(NO_3)_2$, and by urea, $(NH_4)_2SO_4$, and $Ca(NO_3)_2$ in 1919, the nitrogen content as such being approximately the same in all media. Each of these sources of nitrogen was used in duplicate series, to one of which glucose was added. (In 1919 NH_4NO_3, $(NH_4)_2SO_4$, and $Ca(NO_3)_2$ were also used in series to which mannite was added.) No change was made in the other constituents of the full nutrient solution, so that in all series these salts were present in the proportions indicated above. The 1917–18 experiment included the following series, arranged according to nitrogen sources and presence or absence of glucose:

Series 1. Glycocoll (1.07 gr. per liter)—no glucose.
Series 1*A*. Same solution, with 1 percent glucose.
Series 2. Asparagine (0.942 gr. per liter)—no glucose.
Series 2*A*. Same solution, with 1 percent glucose.
Series 3. Ammonium sulphate (0.942 gr. per liter)—no glucose.
Series 3*A*. Same solution, with 1 percent glucose.
Series 4. Ammonium nitrate (0.5 gr. per liter)—no glucose.
Series 4*A*. Same solution, with 1 percent glucose.
Series 5. Calcium nitrate [$Ca(NO_3)_2.4H_2O$, 1.475 gr. per liter]—no glucose.
Series 5*A*. Same solution, with 1 percent glucose.

In making up the media for the above series, sufficient nutrient solution with any one nitrogen source was prepared to supply both the series without glucose and the series with glucose. For these solutions the required amounts of the several constituents, with the exception of ferrous sulphate, were weighed out individually and dissolved in the proper volume of distilled water. (A stock solution of ferrous sulphate was prepared by dissolving

0.1 gr. in 2,000 cc. of distilled water, 50 cc. of which were used in the preparation of each liter of nutrient solution.) The solution thus prepared was divided into two equal quantities in large flasks, 1.5 percent agar being added to each, and 1 percent glucose to one portion. The total nitrogen content of the medium with any one nitrogen source should therefore be the same per unit weight in the two series, with and without glucose, except for traces of nitrogen in the glucose or for slight discrepancies in the actual amount or composition of agar added. The nitrogen-containing compounds were not dried to constant weight nor was the agar purified in any way, all substances being added to the solution directly from the stock bottles. The chemicals used were Baker's "analyzed" and Merck's "highest purity"; the agar was of the kind known as "Difco bacto."

The total nitrogen content of each culture medium was determined by actual analysis of weighed portions of that medium. It is obvious that by this method any nitrogen introduced with the agar or as impurities with the glucose would be completely accounted for.[1]

The 1919 experiment was a partial duplication and an extension of that of the previous year and included the following series:

Series 6. Urea (0.375 gr. per liter)—without glucose.
Series 6*A*. Same solution, with 1 percent glucose.
Series 7*A*. Ammonium sulphate (0.621 gr. per liter), with 1 percent glucose.
Series 7*B*. Ammonium sulphate (as above), with 1 percent mannite.
Series 8. Ammonium nitrate (0.5 gr. per liter)—no glucose or mannite.
Series 8*A*. Same solution, with 1 percent glucose.
Series 8*B*. Same solution as series 8, with 1 percent mannite.
Series 9. Calcium nitrate [$Ca(NO_3)_2.4H_2O$, 1.475 gr. per liter]—no glucose or mannite.
Series 9*A*. Same solution, with 1 percent glucose.
Series 9*B*. Same solution as series 9, with 1 percent mannite.

Each complete solution was placed in the autoclav under 15 pounds' pressure until the agar was dissolved. The solution was then filtered through absorbent cotton; the filtrate was free from sediment.

Introduction of the Agar.—The Kjeldahl flasks were cleaned in the usual way and dried in the hot air oven. On removal from the oven, cotton plugs were inserted in the mouths of the flasks to prevent the entrance of dust. Each flask was numbered by means of a carborundum point and weighed to within 0.05 gram on a Mackenzie one-pan balance, the cotton plug being removed only during the weighing. The flasks were then stored in clean, dry boxes until required.

As soon as the medium for a series was prepared the required number of flasks (11 in 1917–18, 24 in 1919) were arranged in one of the special wooden racks (see fig. 1, Plate I) and 150 cc. of the hot agar solution was added to each flask. The flasks were then immediately weighed in the order in which the agar was introduced. The mouths of the flasks remained plugged with cotton except during the actual processes of introducing the agar and of weighing. Although water vapor condensed on the walls and necks of flasks as the agar cooled, the use, with a number of flasks, of rubber stoppers instead of cotton plugs, demonstrated that there was no detectable loss of water vapor through the cotton plug during the interval between the introduction of the agar solution and the weighing. Thus the actual weight of medium in each flask was known, and, as will be seen from the tables, differences resulted of one or two grams in the weight of approximately equal volumes between the first and last flask of a series to receive the medium, due to the cooling of the agar during the processes involved.

---

[1] Numerous analyses of the agar showed the nitrogen content to be about 1 mg. for the amount present in each culture flask. It will be noticed that the analyses for the total nitrogen of the media may not have yielded exactly the calculated amounts, because of the moisture present in the nitrogen-containing compounds.

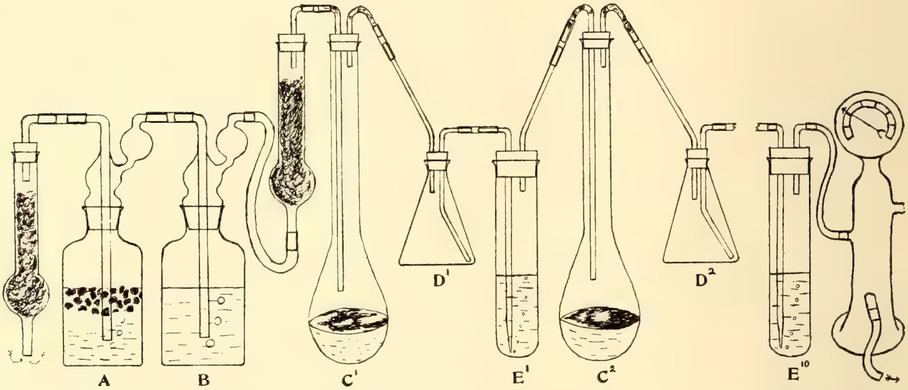
After the second weighing each flask was provided with a two-hole rubber stopper carrying intake and outlet glass tubes, the outer arms of these tubes being adjusted so as to be readily connected in series by means of rubber tubing. Long cotton plugs were loosely adjusted in the bore of each outer arm. The flasks were sterilized at 15 pounds' pressure for 20 minutes, the stoppers resting lightly in the mouths of the flasks but being tightly adjusted upon removal from the autoclav, the hands being moistened with alcohol for this operation. The flasks were allowed to cool in a dust-proof case.

Inoculation.—The inoculations were made in the laboratory under a glass dust shield open on one side only. The inoculum consisted of a suspension of the algal cells in a test tube of sterilized nutrient solution minus combined nitrogen. Special cultures on hard (2 percent) agar were prepared, so that in making the suspension no agar was introduced with the cells. The tube was thoroughly shaken to secure a uniform suspension of the inoculum, of which ten drops were added to each flask in the 1917–18 experiment and one cubic centimeter was similarly added in 1919. In the former experiment four species were used and two flasks in each series were inoculated with the same species, three flasks of each medium remaining uninoculated as checks. In 1919 seven species were used, three flasks of each series being inoculated with each species with the exception of species no. 3 and no. 7, in which cases only two flasks of any one medium were inoculated; three flasks of each series remained uninoculated as checks.

After the rubber stoppers were tightly fitted in the flasks, melted paraffin was run in around the flared neck, and the stopper and a portion of the neck of each flask were covered with sterilized cotton. During the two experiments eighteen contaminations occurred out of a total of 340 flasks.

Aeration.—The intake and delivery tubes of the flasks of each series were connected with rubber tubing for the purpose of aeration. In making connections the free ends of the glass tubes were painted with 95 percent alcohol, which was used also in washing out the bore of the rubber tubing.

In setting up the first experiment it was thought that in the process of aeration a loss of nitrogen from the medium in the form of ammonia might occur, especially in view of the fact that the medium was slightly alkaline, so that a tube of acid was inserted in the series just beyond each culture flask. For this purpose large test tubes, 200 x 25 mm., and containing 25 cc. of standardized N/10 sulphuric acid were used. As a precaution against the backflow of this acid into the cultures, small Erlenmeyer flasks of 180 cc. capacity were placed between each culture flask and its corresponding acid tube. The arrangement of the apparatus can readily be understood by consulting text figure 1. Two gas-washing bottles, *A* and *B*, were placed at the head of each series; *A* contained 30 percent sulphuric acid and a quantity of pumice stone; *B* contained sterilized distilled water. Air entered the series through a calcium chloride tube filled with cotton, was washed free of ammonia by the acid in *A* and was moistened by the water in *B*. Oxides of nitrogen would also be removed by the water. Before entering the culture flask *C′* the air passed through a second calcium chloride tube containing sterilized cotton. The intake tube of each culture extended to within an inch or so of the surface of the agar medium, whereas the delivery tube merely penetrated the rubber stopper, so that in the process of aeration the air above the agar surface was completely changed. After leaving the culture flask, the air passed through the safety flask *D′* and bubbled through the acid in the adjoining test tube *E′*. The intake tube of the latter was drawn out to a fine point which extended to the very bottom of the test tube, so that only very small bubbles were formed, insuring a thorough washing of the air before it passed into the next culture flask of the series. The delivery tube of the last acid tube in the series was connected to a filter pump, by means of which the air was drawn through the whole series at once.



TEXT FIG. 1. Detail of a portion of one of the series of 1917–18. Explanation in the text.

When the ten series of the first experiment had been completely assembled, the delivery tubes at the end of each rack were connected in a single series by means of T-tubes so that aeration of all ten series could be accomplished by one operation. Each of these delivery tubes was provided with a screw clamp which was kept tightly closed except during the process of aeration, when it served to control the volume of air passing through the series. With a few exceptions aeration was continued for an hour every morning, it being considered that this would entirely replace the air in the apparatus. The 1917–18 experiment as completely assembled is shown in figure 1, Plate I.

At the end of the first experiment, titrations of the contents of the acid tubes showed that no appreciable change had taken place in the concentration in any instance. It was assumed, therefore, that with these species and with the conditions realized in the experiment there was no loss of ammonia from the culture flasks. For this reason the tubes of acid were omitted from the second experiment, and in the process of aeration the air passed directly from one culture flask to the next in the series. Because of the expansion of the air in the culture flasks during the hot summer days, the liquids in the gas-washing bottles were frequently forced out through the intake tubes of these bottles,—making it necessary to place safety flasks outside the acid bottle, and between the acid and water bottles, to receive those liquids. During the process of eration the acid and water were drawn back into the proper bottles so that no air ever entered the series without first passing through the liquids. Aeration was continued for an hour every other morning during the growing period.

Cultural Conditions.—Soon after the inoculation of the culture flasks the ten racks were transferred to the greenhouse where more uniform conditions of light and temperature prevailed than in the laboratory. Since preliminary tests showed that agar cultures of the organisms used were soon killed by direct sunlight, the bench occupied by the apparatus was covered with a canopy of black cloth, which reduced the actinic light intensity to about one eighth that of the normal greenhouse illumination. On cloudy days, however, this canopy was rolled up on both sides, thus permitting better illumination; on clear days the west side was open during the morning only, and during the afternoon the east side only was exposed. The arrangement of the apparatus as finally assembled in the greenhouse is shown in figure 2, Plate I, which is a photograph of the 1919 experiment.

GROWTH OF THE CULTURES

Length of Growing Period.—The approximate length, in days, of the growing period of each series is indicated in the headings of the tables which follow. Inoculations in the first experiment were completed on August 31, 1917, and the analyses were begun in April, 1918. The final inoculations of the second experiment were made in May, 1919, and analyses were started in November of the same year.

Method of Recording Growth.—Records of the growth of the cultures were made at intervals of three or four weeks. These consisted of written notes comparing the development of the different species on the same medium, and the difference in amount of growth of the same species on the ten different media. Charts were also prepared at about monthly intervals showing the growth in each culture flask by means of colored crayons. These were found very helpful in making growth comparisons, as they present to the eye at once the relative development in every flask.

Since it was the plan of the experiments to analyze the entire contents of the culture flasks at the end of the growing period, it was not found advisable to attempt any actual weight determinations of the "crop" produced on the various media by the different species. Experiments with solution cultures are in progress now from which it is hoped some accurate data may be secured, showing the actual amounts of growth produced on different media and what relation the dry weight of algal material produced bears to free nitrogen assimilation. From a comparison of the written notes and colored charts, however, the following general statements may be made.

1917–18 Experiment.—In this experiment there was a remarkable similarity in the amount of development of all four species on any one of the media used. Only in a few cases did there appear to be any marked specific differences in the reactions of the organisms to the medium. In general, the presence of glucose resulted in a vigorous and rapid development of all species, irrespective of the nitrogen source.

Series 1–3. The relative growth of all the cultures is indicated in the tables which follow, by means of plus signs. Since no fixation occurred in the series in which the nitrogen was supplied as ammonium sulphate or in the organic forms used, the detailed observations of these series are omitted. The results on the nitrate media, however, were so striking that a more detailed account of the growth on these media is here presented.

Series 4 (ammonium nitrate, without glucose). Growth was slow but steady in all cases. Species nos. 1, 5, and 6 continued healthy to the last, giving "very fair" growths. The growth of species no. 2 was "fair," but the cultures were dead at the end of the experiment.

Series 4A (ammonium nitrate, with 1 percent glucose). All species started with very vigorous growths, the effect of the presence of glucose being very evident. Species no. 1 produced a "luxuriant" growth at first, but after three months began to deteriorate, turning brown over most of the surface. Before growth had completely ceased, however, both cultures of this species began to revive, and by the end of the fourth month were again bright green. The cultures then slowly waned a second time, only small portions remaining green at the end of the experiment. Species nos. 2, 5, and 6 grew steadily from the start and remained healthy, no. 2 producing a "luxuriant" growth and nos. 5 and 6 "very good" growths.

Series 5 (calcium nitrate, without glucose). The growth was very slow with all species, but all remained healthy. Total growth of species no. 2 was "fair," while for nos. 1, 5, and 6 it was "very fair."

Series 5A (calcium nitrate, with 1 percent glucose). All four species gave vigorous growths on this medium, continuing healthy to the end of the experiment. The effect of the presence of glucose was apparent from the start. The growth of species no. 2 was "luxuriant" and appeared slightly better than the others, all of which were "very good."

1919 Experiment.—So far as this experiment duplicated the previous one, the same general type of growth resulted. The presence of glucose in the medium markedly stimulated the development of all species, irrespective of the source of combined nitrogen. It is also true, however, that death of the cultures always occurred first on the media containing glucose. The presence of mannite apparently had no effect by way of increasing the rapidity or amount of growth of any of the species on any of the three media to which this compound was added. The amount of growth produced on these media appeared practically the same as produced by the same organism with the same source of nitrogen but without either glucose or mannite.

Series 6 and 7. As in the previous experiment, no fixation occurred where combined nitrogen was supplied in an organic form or as ammonium sulphate; growth observations for these media are therefore omitted.

Series 8 (ammonium nitrate, without glucose or mannite). A slow, steady growth resulted, as in the previous experiment. At analysis all cultures were healthy. Species nos. 1, 6, and 11 produced "very fair" growths; nos. 2 and 5, "fair." Species nos. 3 and 7 were not grown on this medium.

Series 8A (ammonium nitrate, with 1 percent glucose). All species grew very vigorously at first, but deterioration soon set in and by the end of one month all cultures of nos. 1 and 2 were dead, after a "fair" growth, and species nos. 3 and 6 were rapidly waning. The cultures of no. 3 died after a "fair" growth. One culture of no. 6 also died, but the two others revived and remained healthy to the end of the experiment, giving "very good" growths. Species no. 11 gave a "luxuriant" growth, but at the end of the experiment was turning brown. Nos. 5 and 7 remained healthy throughout the growing period, both producing "very good" growths.

Series 8B (ammonium nitrate, with 1 percent mannite). Growth on this medium was slow, and in general very much as in series 8. All cultures remained healthy at the end of the experiment. Species nos. 1, 3, 6, and 11 gave "very fair" growths, the development being somewhat better than with species nos. 2, 5, and 7.

Series 9 (calcium nitrate, without glucose or mannite). Growth was very slow, and strikingly like that in series 8. All cultures remained healthy, species no. 6 giving a "good" growth, nos. 1 and 11 "very fair" growths, and nos. 2 and 5 "fair." Species nos. 3 and 7 were not included in this series.

Series 9A (calcium nitrate, with 1 percent glucose). Development was very vigorous from the start, all cultures soon producing a complete coat over the agar surface. At the end of a month, species nos. 3, 5, 6, and 11 began to turn yellow. The deterioration, however, did not progress far, and after another month or two all cultures were again green, remaining healthy until analysis. Species nos. 1, 2, and 7 remained healthy throughout the entire growing period. At the end of the experiment a "luxuriant" growth had resulted with species nos. 2 and 11, "very good" growth with nos. 1, 5, and 6, "good" with no. 3, and "slight" with no. 7.

Series 9B (calcium nitrate, with 1 percent mannite). The development was very similar to that in series 9, being slow but steady. All cultures remained healthy to the end. Species nos. 1 and 11 produced "very fair" growths, nos. 2, 5, 6, and 3 "fair," and no. 7 only "slight."

It should be noted that in the 1917–18 experiment the development of the four species on the two nitrate media with glucose (series 4*A* and 5*A*) was very similar, the growth being either "luxuriant" or "very good" in all cases. Of the two, however, the cultures of series 5A appeared somewhat the better. In striking contrast with this condition was the growth of the various species on similar media of the 1919 experiment (series 8*A* and 9*A*). The species which gave such good growths on ammonium nitrate with glucose (series 4*A*) in 1917–18 showed scarcely any development on this medium in 1919 (series 8*A*), with the possible exception of species no. 5. However, on calcium nitrate with glucose (series 9*A*) the growth of these species was practically the same as was secured on the similar medium of 1917–18 (series 5*A*). Even though a few of the flasks of series 8*A* were reinoculated, the growth continued poor; likewise, "reserve" flasks of this medium which were not introduced in the series at the beginning of the experiment gave very similar growths of species nos. 1 and 2 when inoculated a few months before the end of the growing period. This difference in the amount of growth produced in the two experiments may possibly be related to the difference in the seasons during which the experiments were conducted.

ANALYSES

At the end of a growing period of from six to eight months the cultures and checks were analyzed for total nitrogen content. After making a final record of the growth and condition of the cultures, a small loop of the algal material from each flask was transferred to a drop of sterilized nutrient solution and examined microscopically. In 1918 transfers were also made from each culture to nutrient agar containing 1 percent glucose, and to the same medium made acid by the addition of 1 percent hydrochloric acid. Two tubes of each of these media were inoculated from each culture flask, but in no case did contaminations appear that were not apparent from the microscopical examination. This precaution was therefore omitted in 1919, the microscopical examination being relied upon to detect contaminations not apparent to the naked eye. In all cases, however, such contaminations as occurred were perfectly evident from macroscopic examinations, the majority of them appearing soon after inoculation.

After considerable preliminary work with total nitrogen determinations, the Gunning-Kjeldahl method was adopted for media free from nitrates, and in the presence of nitrates the Förster modification of this method was used. In the former method the digestion mixture consists of a solution of 20 grams phosphorus pentoxide (P_2O_5) in 500 cc. sulphuric acid of sp. gr. 1.84, 20 cc. of this solution being added to each culture flask. After the addition of 10 grams potassium sulphate to each, the flasks were heated slowly over a low flame. The presence of the agar made the digestions particularly trying, as it was necessary to watch the flasks constantly at the beginning of the process in order to prevent the contents from foaming up into the necks. It was found advisable to start with a very low flame and to shake the flasks occasionally until sufficient agar had gone into solution to allow the ready escape of bubbles from below. At this point the full flame was used, and the water boiled off vigorously until foaming began. The flame was then turned very low again for about 30 minutes or until the appearance of dense, white fumes, at which time foaming gradually ceased. The fire was then slowly increased to full capacity and the digestion continued for 15 or 20 minutes after a clear liquid resulted. About 1½ hours were required for digestion after foaming ceased. In all cases the flasks at the end of the digestion were perfectly clean and the liquid was entirely transparent.

The distillation was carried out in the usual way. About 150–200 cc. of distilled water was added to each flask when cool, the neck of the flask being thoroughly washed down in the process. The solution was made alkaline with 50 cc. concentrated sodium hydroxide, and, after the addition of a gram of granulated zinc, the flask was immediately connected to the still. The ammonia was distilled over through block tin tubes into a 500-cc. Erlenmeyer flask containing 30 cc. standardized tenth-normal sulphuric acid, diluted with enough distilled water to cover completely the end of the delivery tube. Standard traps were used between the Kjeldahl flask and the condenser. Distillation was continued until the contents of the flask began to "bump." About 125 cc. of water was distilled over in this process, and it was usually completed in about 40 minutes. During the latter part of the distillation the receiving flask was drawn away from the still sufficiently to uncover the end of the delivery tube, the inside walls of which were washed down by the remaining distillate. At the end of the distillation the delivery tube, as well as the inside wall of the receiving flask, was washed down with a small amount of distilled water. The excess acid was titrated against tenth-normal sodium hydroxide, using cochineal as an indicator.

The Förster modification, employed in analyzing the media containing nitrates, consists essentially in the addition of sodium thiosulphate to the digestion mixture. The procedure was as follows: A considerable part of the water of the medium was boiled off in the presence of 10 cc. of concentrated sulphuric acid, the process being continued until active foaming began. When cool, 20 cc. of the phenol-sulphuric acid digestion mixture (100 gr. phenol in 924 cc. concentrated sulphuric acid) were added and allowed to stand several hours or over night, the mouths of the flasks being covered during this time. Two grams of sodium thiosulphate were then added, and, after the reaction was completed, 1 gram of mercuric oxide, 1 gram of zinc dust, 10 cc. of concentrated sulphuric acid, and finally 10 grams of potassium sulphate were added in the order named. The flasks were heated over a low flame until foaming ceased, when the flame was gradually increased to full intensity. Digestion was continued for about 20 minutes after the liquid became clear, the process requiring about 2½ or 3 hours. The distillation was carried out as with the Gunning method, except that before making the solution alkaline the mercury was precipitated out by the addition of 25 cc. potassium sulphide solution (20 gr. K_2S in 500 cc. H_2O).

Numerous "blank" determinations of the nitrogen content of the reagents used in the two methods were made, the average of these being deducted from the determinations of the cultures and checks. Care was taken to use uniform reagents for the analyses of each series.

In the preliminary determinations no difficulty was experienced with the Gunning method in securing complete recovery of the nitrogen from solutions of urea or ammonium sulphate. In the presence of agar or of agar and glucose the usual trouble with foaming was encountered, but the determinations checked readily within the limits of experimental error.

The recovery of total nitrogen, including nitrates in the presence of agar, was more difficult, but consistent results were obtained with the Förster method as outlined above. It was found necessary to allow the digestion mixture to stand a considerable length of time (usually over night) in contact with the dissolved and partially concentrated agar medium before the addition of the other reagents. Following the experience of Duggar and Davis (1916), it was also found advisable to permit the flasks to stand about an hour after the addition of the sodium thiosulphate. The low results obtained when the digestions are completed more quickly may possibly be due to the rather extensive dilution of the reagents by the water of the culture medium and the consequent slower reduction of the nitrates. Tables A and B show some of the results obtained in the preliminary determinations.

TABLE A. *Recovery of total nitrogen from ammonium nitrate by Förster method*
Solution = 1.4285 grams dry NH_4NO_3 in 250 cc. distilled water

10 Cc. Solution Made Alkaline and Distilled	Mg. N as NH_3 Recovered	Mg. N as NH_3 by Calculation	Mg. Difference
1	9.980	10.000	−0.020
2	9.896	10.000	−0.104
3	9.980	10.000	−0.020
10 Cc. Solution Digested by Föster Method	**Mg. Total Nitrogen Recovered**	**Mg. Total Nitrogen by Calculation**	**Mg. Difference**
1	19.812	20.000	−0.198
2	19.540	20.000	−0.460
3	19.931	20.000	−0.069

TABLE B. *Recovery of total nitrogen from ammonium nitrate in the presence of agar agar by Förster method*

Solution of NH_4NO_3. Total calculated N in 10 cc. = 26.252 mg.

Total Mg. N Found in 10 Cc. Solution	Total Mg. N Found in 10 Cc. Solution Plus 2 Grams Agar	Mg. N Found in 2 Grams Difco Agar	Mg. N Recovered
26.268	27.760	1.826	25.993
26.219	27.372	1.741	25.615
26.024	26.994	1.703	25.237

It is apparent from table B that the agar interferes somewhat with the complete recovery of nitrogen. However, as the amount of agar in all series was the same in both checks and cultures, the results, though perhaps low, are still strictly comparable.

RESULTS

The results of the analyses of the cultures are presented in tables 1–9B. These are numbered to correspond with the series, each series representing a single medium. As already explained, the only constituent of the mineral nutrient solution which was varied in the different media was the nitrogen source; the latter is indicated in the heading of each table. The presence or absence of glucose or mannite is indicated in the sub-heading. In the third column the relative growth in each culture flask is shown by means of plus marks; in nearly all cases the growth in the two or three flasks of a series inoculated with the same organism was practically the same, but a few exceptions may be noted. In the fourth column is given the total nitrogen (in milligrams) found in each flask. This figure represents the total nitrogen calculated from the titration, minus the average amount of nitrogen in the blanks. The other columns are self-explanatory.

TABLES 1 AND 1A. *Results of analyses of mineral nutrient agar cultures of green algae. Experiment of 1917–1918. Growth period, 230 days. Nitrogen source,* GLYCOCOLL

1. *Medium without glucose*

Culture		Growth	Total Mg. N Found	Wt. of Medium, Grams	Mg. N per 100 Gr. Medium	Average	Mg. Loss or Gain, 100 Gr.
No.	Species						
–	Check	Con.*	32.655	145.90	22.382	22.214	
–	Check	Ster.	32.389	147.10	22.018		
–	Check	Ster.	32.796	147.45	22.242		
1	*Chlorella vulgaris* Beyr.	+++†	31.823	144.90	21.962	22.017	–0.197
1		+++	32.036	145.15	22.071		
2	*Stichococcus* sp.	++++*	31.859	145.30	22.926	22.020	–0.194
2		+++	32.230	145.75	22.113		
5	*Chlorella* (?) sp.	+++	31.965	146.15	21.871	22.028	–0.186
5		+++*	32.478	146.40	22.184		
6	*Scenedesmus* (?) sp.	+++	32.354	146.70	22.055	21.930	–0.284
6		+++	32.053	147.00	21.805		
	Average all cultures		.....	.....	.....	21.999	–0.215

1A. *Same medium, with 1 percent glucose*

Culture		Growth	Total Mg. N Found	Wt. of Medium, Grams	Mg. N per 100 Gr. Medium	Average	Mg. Loss or Gain, 100 Gr.
No.	Species						
–	Check	Ster.	31.646	146.70	21.572	21.502	
–	Check	Ster.	31.841	148.00	21.514		
–	Check	Ster.	31.735	148.15	21.421		
1	*Chlorella vulgaris* Beyr.	+++++	31.010	146.55	21.160	21.466	–0.056
1		+++++	31.859	146.60	21.732		
2	*Stichococcus* sp.	+++++	31.438	146.55	21.452	21.450	–0.052
2		+++++	31.452	146.65	21.447		
5	*Chlorella* (?) sp.	+++++	30.656	147.20	20.826	21.055	–0.447
5		+++++	31.328	147.20	21.283		
6	*Scenedesmus* (?) sp.	+++++	31.540	147.50	21.383	21.378	–0.124
6		+++++	31.611	147.90	21.373		
	Average all cultures		.....	.....	.....	21.332	–0.170

TABLES 2 AND 2A. *Results of analyses of mineral nutrient agar cultures of green algae. Experiment of 1917–1918. Growth period, 234 days. Nitrogen source,* ASPARAGINE

2. *Medium without glucose*

Culture		Growth	Total Mg. N Found	Wt. of Medium, Grams	Mg. N per 100 Gr. Medium	Average	Mg. Loss or Gain, 100 Gr.
No.	Species						
–	Check	Ster.	27.808	146.50	18.982	19.031	
–	Check	Ster.	28.162	147.25	19.125		
–	Check	Ster.	27.967	147.30	18.987		
1	*Chlorella vulgaris* Beyr.	++	27.861	146.20	19.057	18.966	–0.065
1		++	27.614	146.30	18.875		
2	*Stichococcus* sp.	++	28.410	146.30	19.419	19.192	+0.161
2		++	27.755	146.35	18.965		
5	*Chlorella* (?) sp.	+++	27.667	146.50	18.885	18.806	–0.225
5		+++	27.454	146.60	18.727		
6	*Scenedesmus* (?) sp.	+++	27.614	146.90	18.798	18.783	–0.248
6		+++	27.578	146.95	18.767		
	Average all cultures		.....	.....	.....	18.937	–0.094

* Checks or cultures which were contaminated are designated with an asterisk.

† Relative growth of the cultures is indicated by plus signs, as follows: + = "slight," ++ = "fair," +++ = "very fair," ++++ = "good," +++++ = "very good," ++++++ = "luxuriant."

2A. *Same medium, with 1 percent glucose*

–	Check	Ster.	27.260	147.05	18.538	18.655	
–	Check	Ster.	Lost	147.70	. . . . .		
–	Check	Ster.	27.773	147.95	18.772		
1	*Chlorella vulgaris* Beyr.	+++++	26.924	146.95	18.322	18.344	−0.311
1		+++++	26.941	146.70	18.365		
2	*Stichococcus* sp.	+++++	27.277	146.80	18.581	18.443	−0.212
2		+++++	26.888	146.90	18.304		
5	*Chlorella* (?) sp.	+++++	26.959	147.25	18.308	18.119	−0.536
5		+++++	26.393	147.20	17.930		
6	*Scenedesmus* (?) sp.	+++++	26.835	147.40	18.205	18.289	−0.366
6		+++++	27.154	147.80	18.372		
	Average all cultures		. . . . .	. . . . .	. . . . .	18.299	−0.356

TABLES 3 AND 3A. *Results of analyses of mineral nutrient agar cultures of green algae. Experiment of 1917–18. Growth period, 226 days. Nitrogen source,* AMMONIUM SULPHATE

3. *Medium without glucose*

Culture		Growth	Total Mg. N Found	Wt. of Medium, Grams	Mg. N per 100 Gr. Medium	Average	Mg. Loss or Gain, 100 Gr.
No.	Species						
–	Check	Ster.	29.842	146.75	20.335	20.441	
–	Check	Ster.	30.338	147.65	20.547		
1	*Chlorella vulgaris* Beyr.	+++	29.719	145.50	20.425	20.388	−0.053
1		+++	29.701	145.95	20.350		
2	*Stichococcus* sp.	++	30.055	145.80	20.614	20.591	+0.150
2		++	30.090	146.30	20.567		
5	*Chlorella* (?) sp.	++	30.055	147.00	20.445	20.658	+0.217
5		+++*	30.691	147.05	20.871		
6	*Scenedesmus* (?) sp.	+++	30.090	147.20	20.441	20.494	+0.053
6		+++	30.284	147.40	20.546		
	Average all cultures		. . . . .	. . . . .	. . . . .	20.533	+0.092

3A. *Same medium, with 1 percent glucose*

–	Check	Ster.	29.400	147.10	19.986	19.957	
–	Check	Ster.	29.233	148.05	19.739		
–	Check	Ster.	29.825	148.05	20.145		
1	*Chlorella vulgaris* Beyr.	(++++)†	28.834	146.75	19.648	19.657	−0.300
1		(++++)	28.869	146.80	19.666		
2	*Stichococcus* sp.	(++++)	29.365	146.85	19.996	19.836	−0.121
2		(++++)	28.923	147.00	19.675		
5	*Chlorella* (?) sp.	++++++	29.188	147.35	19.809	19.776	−0.181
5		++++++	29.082	147.30	19.743		
6	*Scenedesmus* (?) sp.	(+++)	28.746	147.75	19.456	19.261	−0.696
6		(+++)	28.180	147.80	19.066		
	Average all cultures		. . . . .	. . . . .	. . . . .	19.634	−0.323

† Plus signs enclosed in parentheses indicate that the culture was dead at the time of analysis.

TABLES 4 AND 4A. *Results of analyses of mineral nutrient agar cultures of green algae. Experiment of 1917–18. Growth period, 270 days. Nitrogen source,* AMMONIUM NITRATE

4. *Medium without glucose*

Culture		Growth	Total Mg. N Found	Wt. of Medium, Grams	Mg. N per 100 Gr. Medium	Average	Mg. Loss or Gain, 100 Gr.	Gain Percent
No.	Species							
–	Check	Ster.	23.569	146.90	16.044	15.788		
–	Check	Con.*	23.885	147.95	16.144			
–	Check	Con.*	22.445	148.25	15.175			
1	*Chlorella vulgaris*	+++*	22.410	146.20	15.328	15.758	−0.030	
1	Beyr.	+++*	23.674	146.25	16.188			
2	*Stichococcus* sp.	(++)	24.921	146.45	17.017	16.542	+0.754	4.7
2		(++)	23.569	146.70	16.066			
5	*Chlorella* (?) sp.	++++	25.729	146.90	17.515	17.027	+1.239	7.8
5		+++	24.377	147.40	16.538			
6	*Scenedesmus* (?) sp.	+++	25.940	147.60	17.574	17.574	+1.786	11.3
6		++	Lost	147.75	.....			
	Average all cultures		.....	.....	.....	16.725	+0.937	5.9

4A. *Same medium, with 1 percent glucose*

Culture		Growth	Total Mg. N Found	Wt. of Medium, Grams	Mg. N per 100 Gr. Medium	Average	Mg. Loss or Gain, 100 Gr.	Gain Percent
No.	Species							
–	Check	Ster.	24.323	147.75	15.794	16.032		
–	Check	Ster.	23.446	148.60	15.778			
–	Check	Ster.	24.095	145.90	16.525			
1	*Chlorella vulgaris*	(++++)	32.747	146.90	21.978	22.136	+6.104	38.
1	Beyr.	(+++)+	32.817	147.20	22.294			
2	*Stichococcus*	++++++	32.269	147.10	21.987	21.910	+5.878	36.
2		++++++	32.203	147.50	21.832			
5	*Chlorella* (?) sp.	+++++	33.291	147.90	22.509	22.394	+6.362	39.6
5		+++++	33.028	148.25	22.278			
6	*Scenedesmus* (?) sp.	+++++	33.624	148.25	22.681	22.179	+6.147	38.3
6		+++++	32.168	148.40	21.676			
	Average all cultures........		.....	.....	.....	22.155	+6.123	38.

TABLES 5 AND 5A. *Results of analyses of mineral nutrient agar cultures of green algae. Experiment of 1917–18. Growth period, 297 days. Nitrogen source,* CALCIUM NITRATE

5. *Medium without glucose*

Culture		Growth	Total Mg. N Found	Wt. of Medium, Grams	Mg. N per 100 Gr. Medium	Average	Mg. Loss or Gain, 100 Gr.	Gain Percent
No.	Species							
–	Check	Ster.	24.850	147.35	(16.903)	†16.903		
–	Check (Loss)	Ster.	21.182	148.25	(14.289)			
1	*Chlorella vulgaris*	+++	23.797	145.65	16.338	16.258	−0.645	
1	Beyr.	+++	23.586	145.80	16.177			
2	*Stichococcus* sp.	++	23.990	146.05	16.425	16.368	−0.535	
2		++	23.902	146.55	16.310			
5	*Chlorella* (?) sp.	+++	27.219	147.30	18.479	18.755	+1.852	11.
5		+++	28.079	147.55	19.030			
6	*Scenedesmus* (?) sp.	+++	27.079	147.80	18.322	16.565	−0.338	
6		+++	24.078	148.00	16.269			
6		+++	23.393	148.25	15.105			
	Average all cultures		.....	.....	.....	16.987	+0.084	

5A. *Same medium, with 1 percent glucose*

–	Check (Loss)	Ster.	22.603	148.50	15.221	†16.903		
–	Check (Loss)	Ster.	18.360	147.40	12.456			
1	*Chlorella vulgaris* Beyr.	+++++	35.274	147.05	23.988	24.430	+7.527	45.1
1		+++++	36.572	147.05	24.871			
2	*Stichococcus* sp.	++++++	36.029	147.25	24.468	23.604	+6.701	39.6
2		++++++	33.519	147.40	22.740			
5	*Chlorella* (?) sp.	+++++	35.327	147.80	24.402	24.402	+7.499	44.3
5		+++++	Lost	147.90	.....			
6	*Scenedesmus* (?) sp.	+++++	35.502	148.25	23.947	23.956	+7.053	41.7
6		+++++	34.607	148.80	23.257			
6		++++	36.590	148.35	24.665			
	Average all cultures		.....	.....	.....	24.098	+7.195	42.5

TABLES 6 AND 6A. *Results of analyses of mineral nutrient agar cultures of green algae. Experiment of 1919. Growth period, 165 days. Nitrogen source,* UREA

6. *Medium without glucose*

Culture		Growth	Total Mg. N Found	Wt. of Medium, Grams	Mg. N per 100 Gr. Medium	Average	Mg, Loss or Gain, 100 Gr.
No.	Species						
—	Check	Ster.	25.212	148.05	17.029	17.465	
—	Check	Ster.	26.315	148.30	17.744		
—	Check	Ster.	26.161	148.45	17.623		
1	*Chlorella vulgaris* Beyr.	+++	24.807	148.15	16.745	16.696	−0.769
1		+++	24.779	148.15	16.726		
1		+++	24.583	147.95	16.616		
2	*Stichococcus* sp.		24.667	148.05	16.661	16.714	−0.715
2			24.988	148.05	16.878		
2			24.580	148.05	16.603		
5	*Chlorella* (?) sp.		25.212	148.05	17.029	16.737	−0.728
5			24.779	148.15	16.726		
5			24.388	148.20	16.456		
6	*Scenedesmus* (?) sp.	++	24.480	148.15	16.524	16.831	−0.634
6		++	24.960	148.25	16.836		
6		++	25.407	148.30	17.132		
11	*Chlorella* (?) sp.	++	25.687	148.20	17.333	17.199	−0.266
11		++	25.533	148.30	17.217		
11		++	25.282	148.30	17.048		
	Average all cultures		.....	.....	.....	16.835	−0.630

---

† Because of the use of some old Jena flasks in completing Series 5 and 5A, a number of determinations of checks and cultures were lost. Accordingly, the highest complete check determination is taken for the nitrogen content of the media of these two series.

6A. *Same medium, with 1 percent glucose*

—	Check	Ster.	27.348	148.40	18.429	18.056	
—	Check	Ster.	26.203	148.50	17.645		
—	Check	Ster.	26.943	148.90	18.095		
1	*Chlorella vulgaris* Beyr.	+++++	26.301	148.35	17.729	17.783	−0.273
1		+++++	26.231	148.40	17.676		
1		+++++	26.622	148.35	17.945		
2	*Stichococcus* sp.	++++	25.882	148.45	17.435	17.426	−0.630
2		++++	25.645	148.45	17.275		
2		++++	26.064	148.35	17.569		
5	*Chlorella* (?) sp.	+++++	25.756	148.35	17.366	17.590	−0.466
5		+++++	26.762	148.40	18.034		
5		+++++	25.784	148.45	17.369		
6	*Scenedesmus* (?) sp.	+++++	26.580	148.50	17.899	18.226	+0.170
6		+++++	27.390	148.45	18.451		
6		+++++	27.209	148.45	18.329		
11	*Chlorella* (?) sp.	+++++	26.860	148.45	18.094	18.295	+0.239
11		+++++	27.363	148.45	18.433		
11		+++++	27.306	148.75	18.357		
7	*Protococcus* sp.		26.846	148.70	18.054	18.406	+0.350
7			27.893	148.70	18.758		
3	*Protosiphon botryoides*	+++++	27.069	148.70	18.204	18.258	+0.202
3		+++++	27.265	148.90	18.311		
	Average all cultures		.....	.....	.....	17.998	−0.058

TABLES 7A AND 7B. *Results of analyses of mineral nutrient agar cultures of green algae. Experiment of 1919. Growth period, 175 days. Nitrogen source,* AMMONIUM SULPHATE

7A. *Medium with 1 percent glucose*

Culture		Growth	Total Mg. N Found	Wt. of Medium, Grams	Mg. N per 100 Gr. Medium	Average	Mg. Loss or Gain, 100 Gr.
No.	Species						
—	Check	Ster.	21.385	148.30	14.421	14.462	
—	Check	Ster.	21.986	148.80	14.776		
—	Check	Ster.	21.190	149.35	14.188		
1	*Chlorella vulgaris* Beyr.	(+)	20.729	148.40	13.968	14.084	−0.378
1		(+)	20.799	148.15	14.039		
1		(+)	21.148	148.45	14.246		
2	*Stichococcus* sp.	(+++)	20.561	148.25	13.869	14.176	−0.286
2		(+++)	21.651	147.95	14.634		
2		(+++)	20.799	148.30	14.025		
5	*Chlorella* (?) sp.	+++++	20.380	148.60	13.715	13.925	−0.537
5		+++++	20.771	148.65	13.973		
5		+++++	20.911	148.45	14.086		
6	*Scenedesmus* (?) sp.	(++)	20.310	148.70	13.658	13.784	−0.678
6		(++)	20.799	148.60	13.997		
6		(++)	20.366	148.70	13.696		
11	*Chlorella* (?) sp.	(++++)	20.212	148.90	13.574	13.834	−0.628
11		(++++)	21.162	148.95	14.208		
11		(++++)	20.450	149.05	13.720		
7	*Protococcus* sp.	+++++	21.036	148.95	14.123	14.032	−0.430
7		+++++	20.771	149.00	13.940		
3	*Protosiphon botryoides*	+++	22.098	148.95	14.836	14.398	−0.064
3		+++	20.827	149.20	13.959		
	Average all cultures		.....	.....	.....	14.033	−0.429

7B. *Same nitrogen source. Medium with 1 percent mannite*

—	Check	Ster.	20.520	148.55	13.814	13.993	
—	Check	Ster.	21.162	148.80	14.222		
—	Check	Ster.	13.872	99.50	13.942		
1	*Chlorella vulgaris*	+++*	20.966	148.35	14.133	14.321	+0.328
1	Beyr.	+++	22.070	148.40	14.872		
1		+++	20.729	148.50	13.959		
2	*Stichococcus* sp.	++	20.938	148.65	14.085	13.658	−0.335
2		++	19.570	148.70	13.161		
2		++	20.408	148.65	13.729		
5	*Chlorella* (?) sp.	+++	21.064	148.50	14.185	13.780	−0.213
5		+++	20.603	148.70	13.855		
5		+++	19.765	148.60	13.301		
6	*Scenedesmus* (?) sp.	++	21.637	148.80	14.541	14.061	+0.068
6		++	20.087	148.70	13.508		
6		++	21.008	148.65	14.133		
11	*Chlorella* (?) sp.	++	19.905	148.80	13.377	13.584	−0.409
11		++	20.701	148.90	13.903		
11		++	20.073	149.00	13.472		
7	*Protococcus* sp.	+	20.226	149.00	13.575	13.627	−0.366
7		+	20.380	149.00	13.678		
3	*Protosiphon*	+++	20.757	149.15	13.917	14.040	+0.047
3	*botryoides*	+++	21.134	149.20	14.162		
	Average all cultures		.....	.....	.....	13.867	−0.126

TABLES 8, 8A, AND 8B. *Results of analyses of mineral nutrient agar cultures of green algae. Experiment of 1919. Growth period, 255 days. Nitrogen source,*

AMMONIUM NITRATE

8. *Medium without glucose or mannite*

Culture		Growth	Total Mg. N Found	Wt. of Medium, Grams	Mg. N per 100 Gr. Medium	Average	Mg. Loss or Gain, 100 Gr.
No.	Species						
—	Check	Ster.	26.704	147.45	18.111	17.736	
—	Check	Ster.	26.789	147.95	18.107		
—	Check	Ster.	25.189	148.25	16.991		
1	*Chlorella vulgaris*	+++++*	27.015	147.65	18.292	18.308	+0.672
1	Beyr.	+++	27.029	147.25	18.356		
1		+++	26.958	147.50	18.277		
2	*Stichococcus* sp.	++*	26.576	147.35	18.036	17.989	+0.253
2		+	26.166	147.50	17.740		
2		+	26.888	147.80	18.192		
5	*Chlorella* (?) sp.	+++	25.543	147.65	17.300	17.198	−0.538
5		++	25.413	147.90	17.183		
5		++	25.274	147.70	17.112		
6	*Scenedesmus* (?) sp.	+++	26.619	147.75	18.016	17.866	+0.130
6		+++	26.194	147.95	17.705		
6		+++	26.421	147.80	17.876		
11	*Chlorella* (?) sp.	+++	20.248	147.90	13.690	14.018	−3.718
11		+++	20.615	147.95	13.934		
11		++++	21.380	148.15	14.431		
	Average all cultures (omitting species no. 11)		.....	.....	.....	17.840	+0.104

8A. *Same nitrogen source, medium with 1 percent glucose*

Culture		Growth	Total Mg. N Found	Wt. of Medium, Grams	Mg. N per 100 Gr. Medium	Average	Mg. Loss or Gain, 100 Gr.	Percent Gain
No.	Species							
—	Check	Ster.	25.095	147.70	16.991	17.178		
—	Check	Ster.	25.499	148.00	17.229			
—	Check	Con.*	25.529	147.45	17.314			
1	*Chlorella vulgaris*	(+)	26.774	147.55	18.145	18.272	+1.094	6.
1	Beyr.	(+)	27.369	147.50	18.555			
1		(+)*	26.774	147.80	18.115			
2	*Stichococcus* sp.	(++)	26.180	147.30	17.773	17.417	+0.239	1.4
2		(++)	23.009	147.15	15.636			
2		(++)*	27.822	147.65	18.843			
5	*Chlorella* (?) sp.	+++++	27.161	147.20	18.452	18.973	+1.795	10.
5		+++++	28.658	147.75	19.396			
5		+++++*	28.195	147.85	19.070			
6	*Scenedesmus* (?) sp.	+++++	27.496	147.80	18.604	17.911	+0.733	4.2
6		+++++	24.452	147.85	16.538			
6		+++++	27.496	147.90	18.591			
11	*Chlorella* (?) sp.	+++++	27.358	148.10	18.473	18.293	+1.115	6.5
11		+++++	27.037	148.15	18.250			
11		+++++	26.897	148.15	18.155			
7	*Protococcus* sp.	+++++	28.377	148.10	19.161	19.303	+2.125	12.
7		+++++	28.992	148.25	19.556			
7		+++++	28.433	148.15	19.192			
3	*Protosiphon*	+++	Lost	148.35	.....	18.071	+0.893	5.2
3	*botryoides*	+++	26.619	147.30	18.071			
	Average all cultures		.....	.....	.....	18.320	+1.142	6.6

8B. *Same nitrogen source, medium with 1 percent mannite*

Culture		Growth	Total Mg. N Found	Wt. of Medium, Grams	Mg. N per 100 Gr. Medium	Average	Mg. Loss or Gain, 100 Gr.
No.	Species						
—	Check	Ster.	25.741	146.95	17.517	17.091	
—	Check	Ster.	25.571	148.10	17.266		
—	Check	Con.*	24.184	146.65	16.491		
1	*Chlorella vulgaris*	+++	25.458	146.65	17.360	17.587	+0.496
1	Beyr.	+++	26.350	146.65	17.968		
1		+++	25.033	143.60	17.433		
2	*Stichococcus* sp.	++	24.467	146.80	16.667	16.466	−0.625
2		++	24.000	146.90	16.338		
2		++	24.113	147.10	16.392		
5	*Chlorella* (?) sp.	+++	26.746	147.10	18.182	17.243	+0.152
5		+++	24.368	147.15	16.560		
5		+++	25.047	147.45	16.987		
6	*Scenedesmus* (?) sp.	+++	26.307	147.50	17.835	17.403	+0.312
6		+++	24.269	147.55	16.448		
6		+++	26.449	147.55	17.926		
11	*Chlorella* (?) sp.	++	21.933	147.75	14.845	14.891	−2.200
11		++	22.258	147.80	15.060		
11		++	21.848	147.95	14.767		
7	*Protococcus* sp.	+	24.807	147.85	16.779	17.114	+0.023
7		+	25.840	148.10	17.448		
3	*Protosiphon*	++	25.812	148.15	17.423	16.812	−0.279
3	*botryoides*	++	24.099	148.20	16.200		
	Average all cultures (omitting species no. 11)		.....	.....	.....	17.103	+0.012

TABLES 9, 9A, AND 9B. *Results of analyses of mineral nutrient agar cultures of green algae. Experiment of 1919. Growth period, 235 days. Nitrogen source,* CALCIUM NITRATE

9. *Medium without glucose*

Culture*		Growth	Total Mg. N Found	Wt. of Medium, Grams	Mg. N per 100 Gr. Medium	Average	Mg. Loss or Gain, 100 Gr.	Gain Percent
No.	Species							
—	Check	Ster.	24.202	147.90	16.364	16.755		
—	Check	Ster.	24.146	147.95	16.320			
—	Check	Ster.	26.045	148.15	17.580			
1	*Chlorella vulgaris* Beyr.	+++	25.822	147.25	17.536	17.874	+1.119	6.
1		+++	26.939	147.15	18.307			
1		+++	26.199	147.35	17.780			
2	*Stichococcus* sp.	+	22.735	147.30	15.435	17.058	+0.303	
2		++	26.464	147.45	17.948			
2		+	26.241	147.50	17.791			
5	*Chlorella* (?) sp.	++	27.322	147.60	18.511	18.689	+1.934	11.5
5		++	26.520	147.90	17.931			
5		++	28.978	147.65	19.626			
6	*Scenedesmus* (?) sp.	++	22.875	147.85	15.472	16.522	−0.233	
6		++	24.341	147.90	16.458			
6		++	26.073	147.85	17.635			
11	*Chlorella* (?) sp.	+++	17.121	148.25	11.549	11.439	−5.316	
11		+++	17.749	148.15	11.980			
11		+++	16.046	148.75	10.787			
	Average all cultures (omitting species no. 11)		.....	.....	.....	17.311	+0.556	

9A. *Same nitrogen source, medium with 1 percent glucose*

—	Check	Ster.	23.055	148.70	15.504	15.640		
—	Check	Ster.	22.553	148.85	15.152			
—	Check	Ster.	24.298	149.40	16.264			
1	*Chlorella vulgaris* Beyr.	+++++	34.535	148.70	23.225	22.963	+7.323	47.
1		+++++*	33.948	148.50	22.861			
1		+++++	33.864	148.50	22.804			
2	*Stichococcus* sp.	++++++	35.024	148.50	23.585	22.440	+6.800	43.5
2		++++++	30.820	148.50	20.754			
2		++++++	34.186	148.75	22.982			
5	*Chlorella* (?) sp.	+++++	34.849	148.70	23.436	23.677	+8.037	51.4
5		+++++	35.799	148.90	24.042			
5		+++++	35.071	148.90	23.553			
6	*Scenedesmus* (?) sp.	+++++	34.388	148.75	23.118	23.434	+7.794	49.7
6		+++++	34.975	149.15	23.450			
6		+++++	35.338	148.90	23.733			
11	*Chlorella* (?) sp.	++++++	31.714	148.90	21.299	21.319	+5.679	36.3
11		++++++	32.831	149.00	22.034			
11		++++++	30.750	149.10	20.624			
7	*Protococcus* sp.	+	24.243	149.05	16.265	16.372	+0.732	4.7
7		+	24.578	149.15	16.479			
3	*Protosiphon botryoides*	++++	25.360	149.15	17.003	17.372	+1.732	11.
3		++++	26.477	149.25	17.740			
	Average all cultures		.....	.....	.....	21.082	+5.442	34.8

9B. *Same nitrogen source, medium with 1 percent mannite*

Culture		Growth	Total Mg. N Found	Weight of Medium, Grams	Mg. N per 100 Gr. Medium	Average	Mg. Loss or Gain, 100 Gr.
No.	Species						
—	Check	Ster.	27.865	148.75	18.733	17.728	
—	Check	Ster.	25.360	148.55	17.071		
—	Check	Con.*	25.826	148.60	17.380		
1	*Chlorella vulgaris* Beyr.	+++	22.818	148.70	15.345	15.280	−2.448
1		+++	23.558	148.90	15.821		
1		+++	21.799	148.55	14.675		
2	*Stichococcus* sp.	+++	21.904	148.70	14.730	16.529	−1.199
2		++	24.311	148.70	16.349		
2		++	27.511	148.65	18.507		
5	*Chlorella* (?) sp.	++	26.973	148.60	18.151	17.764	+0.036
5		++	27.029	148.60	18.189		
5		++	25.189	148.60	16.951		
6	*Scenedesmus* (?) sp.	++	19.634	148.55	13.217	15.603	−2.125
6		++	24.326	148.65	16.365		
6		++	25.609	148.65	17.228		
11	*Chlorella* (?) sp.	+++	13.481	148.70	9.066	9.430	−8.298
11		+++	Lost	148.80	.....		
11		+++	14.613	149.20	9.794		
7	*Protococcus* sp.	+	22.251	149.20	14.914	14.914	−2.814
7		+	Lost	149.15	.....		
3	*Protosiphon botryoides*	+++	24.481	149.15	16.414	17.063	−0.665
3		+++	26.293	148.45	17.712		
	Average all cultures (omitting species no. 11)		.....	.....	.....	16.192	−1.536

Discussion

From a study of the foregoing tables it is apparent at once that substantial increases in the total combined nitrogen content of the culture flasks occurred with the media containing nitrate nitrogen and glucose. Fixation on these media was not confined to any one species, most of the forms used showing the ability to utilize the uncombined nitrogen of the air. The cases of Protococcus (species no. 7) and Protosiphon (species no. 3) may be questioned, but it will be noticed that of all the species grown on ammonium nitrate with glucose in the 1919 experiment (table 8A) Protococcus gave the highest gain in nitrogen, and that in the same experiment Protosiphon gave an increase of nearly 2 mg. on calcium nitrate with glucose (table 9A). Although an increase of only 2 mg. is a small amount to base definite conclusions upon, the evidence is certainly in favor of the assumption that these two species also possess the ability to fix nitrogen. As regards species nos. 1, 2, 5, and 6 there can be no question about their ability to fix nitrogen, since the increases with these species range from a fraction of a milligram to over 8 mg. per 100 grams medium, representing additions to the total nitrogen content of the flasks of from 1 to 51 percent. The highest increase noted was in the case of one of the cultures of species no. 5 on calcium nitrate with glucose, in the 1919 experiment (table 9A); this flask showed a total gain of 12.53 mg. over the average of the checks, an increase in nitrogen content of 54 percent. Fixation was secured by these four species in both experiments when grown on similar media and under similar conditions, so that there seems to be no escape from the definite conclusion that these forms do actually use free nitrogen. The amounts of fixation secured by these four species on ammonium nitrate with glucose in 1919 were considerably lower than found in the 1917–18 experiment on a similar medium. As already pointed out, however (page 9), the growth produced on this medium in 1919 was very poor, a factor which, it is believed, accounts for the smaller fixation.

This leaves to be considered the case of species no. 11 (*Chlorella* sp.) which is of considerable interest. In series 8A and 9A (the nitrate media with glucose) substantial increases in the nitrogen content of the cultures occurred, especially on calcium nitrate, where the average of the cultures is more than 5.5 mg. above the checks. This same species, however, on both nitrate media without a carbon source, and on the nitrate media with mannite, showed distinct losses in nitrogen. These losses ranged from 2 to 8 mg. and occurred regularly on the media mentioned with this species only. This species was used only in the 1919 experiment, in which acid tubes were not inserted between adjacent culture flasks, so that there is no means of telling whether the loss occurred as ammonia or as free nitrogen. However, that some sort of process resulting in a loss of introgen has taken place is apparent, and the results suggest that perhaps both the processes of free nitrogen fixation and of denitrification may be going on simultaneously, the former overbalancing the latter when glucose is present.

As regards the use of free nitrogen by the forms investigated on nitrate media to which no carbohydrate energy source was added, the evidence is not conclusive. In 1917–18 gains of from 0.7 to 1.8 mg. per 100 grams medium were recorded for three of the species grown on ammonium nitrate (table 4), and one of these species gave an increase of 1.8 mg. on calcium nitrate without a carbon source (table 5). In 1919 three out of five species showed very slight increases on ammonium nitrate in the absence of a carbon source, while on calcium nitrate without glucose or mannite gains of from 0.3 to 1.9 mg. per 100 grams medium occurred with three species (table 9). As has already been pointed out, however, the growth of all species in the absence of glucose is very slow, and, although the cultures remain in a healthy condition for long periods of time, the actual amount of "crop" produced is slight in comparison with the glucose cultures. That some fixation does take place in the absence of glucose seems probable, in view of the increases cited above, and the assumption that the amount of fixation on one and the same medium is dependent, more or less, on the total amount of growth produced does not seem unwarranted. This phase of the problem is of considerable importance and is one which should be most carefully investigated, as it bears directly on the conditions as they exist in nature. It is entirely possible that the conditions realized in the experiments, under which marked fixation occurred, have merely amplified those already existing in the field, for soluble carbohydrates and a certain amount of nitrates would most certainly be available for the development of these organisms, especially those occurring naturally on soil. If this is actually the case, it is highly probable that fixation occurs also under natural conditions.

The evidence that no fixation occurred on media lacking nitrates is quite conclusive. When nitrogen was supplied as glycocoll, asparagine, urea, or ammonium sulphate, the increase or decrease in the nitrogen content of the culture flasks above or below that of the checks was in all cases less than a milligram, even though the growth, especially on urea with glucose, was as luxuriant as that produced on nitrates in the presence of glucose. The results were uniform for all species grown on media containing these nitrogen sources and were not altered by the presence either of glucose or of mannite, the latter being used only with ammonium sulphate. Although some of the cultures of these series showed higher nitrogen contents than the checks, there were no consistent gains, and in most cases the figures were lower than those of the checks. However, the differences in either case are so small as to be without significance, since most of them are within the usual experimental error.

A comparison of the results presented here with figures secured by a large number of investigators working with the legume nodule bacteria and Azotobacter forms shows that the amount of fixation produced by the algae per unit volume of medium, under the conditions of the experiments, equals, and in some cases surpasses, the average fixation by the colorless forms. The legume bacteria especially give only slight increases when grown in pure culture outside the host plant. Fred (1912) gives a table of the results of 25 investigators, from which it is apparent that the average fixation with this organism is about 0.9 to 2.2 mg. per 100 cc. culture medium. Fred himself secured fixations ranging from 0.4 to 1.58 mg. per 100 cc. culture solution. With the Azotobacter forms the amounts of fixation are considerably larger. *Azotobacter chroococcum*, which has been extensively investigated in pure culture, shows increases ranging from a few milligrams to 20 or 30 mg. per 100 cc. culture medium, the average fixation, however, being usually about 6 or 10 mg. Thus Gerlach and Vogel (1902) report 4 mg. to be the average, while Freudenreich (1903) secured only 1.6 to 2.4 mg. per 100 cc. culture solution, but on gypsum plates the increases were higher, amounting to 16 mg. per 100 cc. solution. Krzemieniewski (1908) secured increases ranging from 1 to 13.5 mg. per 100 cc. solution, an average of 3 to 6 mg. being fixed for each gram of glucose respired. In the presence of humus the ratio was 5 to 10 mg. nitrogen fixed per gram of glucose respired. Hoffmann and Hammer (1910) report fixations of from 4.55 to 14.4 mg. per gram of carbohydrate consumed, and in the same year Krzemieniewska (1910) published results showing increases of from 11 to 18 mg. per 100 cc. culture solution. Remy and Rosing (1911) found from 10 to 20 mg. to be the average fixation per 100 cc. culture, or 2 to 15 mg. per gram of mannite consumed. Perhaps the most extensive experiments with Azotobacter forms are those of Lipman (1903 and 1905), who secured the following gains in nitrogen, each in 100 cc. of culture solution: *A. chroococcum,* 4.42 mg.; *A. beyerinckii,* 2.37 to 6.78 mg.; *A. vinlandii,* 1.67 to 7.90 mg. in 1903, and 8.36 to 20.99 mg. in 1905. The numerous results with mixed cultures and impure soil cultures by many investigators show even wider ranges of fixation, and the conditions of the experiments have been so various that it is difficult to arrive at an average, but in many cases it appears to be between 6 and 10 mg. per 100 grams of culture medium.

Because of the nature of the experiments reported in this paper it is impossible to state in what form the nitrogen is added to the culture. Whether or not the process is connected with that of photosynthesis, the ratio of nitrogen fixed to sugar respired or to the amounts of nitrate absorbed, and the amount fixed per unit dry weight of crop produced are phases of the problem which can be merely mentioned at this time. The solution of these questions will be simplified by the proper development of liquid culture methods, some of which are now in progress.

Since the results obtained are wholly contrary to the generally accepted idea of the relation of green plants to elementary nitrogen, it may be well to point out the conditions under which some of the earlier negative results with the green algae were obtained. The first nitrogen determinations of pure cultures of algae were made by Kossowitsch (1894), who grew a single species of Cystococcus (?) on sand moistened with 20 cc. of a mineral nutrient solution containing calcium nitrate as a source of nitrogen. The cultures, however, grew for only three weeks, because, the author concludes, of the lack of nitrates, only 2.5 mg. nitrogen as nitrate having been supplied. The addition of more calcium nitrate to the cultures caused them to revive, and it was upon this fact that his conclusion was based. However, it is not altogether clear that the lack of growth may not have been due to some other cause, such as deficiency in calcium, only a "trace" of which was present in addition to that added as calcium nitrate. The analyses of the cultures and uninoculated checks clearly showed that there had been no increase in the nitrogen content of the flasks, either in the presence or in the absence of glucose. It is apparent, however, that conditions for a vigorous, long-continued growth were not realized in these experiments, hence it is not to be expected that appreciable increases in nitrogen should occur. As a result of a large number of analyses of pure cultures, Kruger and Schneidewind (1900) concluded that the green algae investigated were unable to use free nitrogen. They grew a large number of forms on a variety of media, using both sand and solution cultures. Two of the media contained nutrient solutions free from combined nitrogen, five contained organic nitrogen supplied in beef extract and peptone, beerwort, or humus, and in the two remaining solutions both ammonium sulphate and sodium nitrate were present; in none of the media was the nitrogen supplied as nitrate only. The cultures were grown in large flasks plugged with cotton, no other provision being made for aeration. No growth resulted on the media free from combined nitrogen; on those to which organic nitrogen sources were added, a vigorous development of the algae resulted in most cases but no increases in nitrogen content were obtained. On the media containing inorganic nitrogen a good growth was obtained only in one experiment with cultures of several species of Stichococcus; in the remaining experiments with these media there was no growth because of the unfavorable reaction of the solution. No increases in nitrogen content were observed, however, in any case. The results of these authors compare very favorably with those presented above in the tables, no fixation having been secured when ammonium sulphate or organic compounds served as nitrogen sources.

In 1903, Charpentier substantiated Kossowitsch's results with Cystococcus. He grew the single species on a bean-glucose-gelatin medium; at the end of 20 days no change in the nitrogen content of the cultures was observed. However, these experiments were neither extensive enough, nor were they continued for sufficient length of time, to be conclusive. The failure to secure fixation may be attributed in this case also to the absence of proper nitrogen sources in the medium.

As already pointed out in the first part of this discussion, the presence of nitrate nitrogen seems to be essential for the process of fixation. In this connection it is interesting to note that the presence of nitrates has been found beneficial for the growth of, and fixation of nitrogen by, Azotobacter forms and the legume bacteria. It has been repeatedly observed that the presence of very slight amounts of nitrates stimulates the development of the nodule bacteria. Thus, Fred and Graul (1916) report that in the presence of small amounts of ammonium nitrate nodules were produced in great numbers on alfalfa and vetch by pure cultures of *Bacillus radicicola*; numerous nodules were formed also in the presence of small amounts of calcium nitrate, but with added amounts the degree of infection declined. In a recent paper, Hills (1918) reports that the presence of small amounts of nitrates exerted an enormous influence on the growth and reproduction of Azotobacter in sterile soil, the amount of fixation in such cultures being also increased in the presence of nitrates. In the case of *Bacillus radicicola* the nitrates stimulated growth and reproduction in sterilized soil, and on agar films the amount of fixation was greater when nitrates were added. The author concludes that this increased fixation may be the result of the increased growth of the organisms where nitrates were supplied. Though such observations as these cannot be claimed to have any direct application to the case of green plants, they are at least suggestive in view of the fact that nitrates have long been regarded as in general the best combined nitrogen source for chlorophyll-bearing plants.

The proof that green plants are capable of fixing free nitrogen is at once of real significance, both from a purely scientific and from a practical standpoint, even though the plants concerned are members of a low order. The question which perhaps at first suggests itself is: Do all green plants possess this ability to use the uncombined nitrogen of the air? With the exception of the legumes, the evidence is almost entirely negative as regards the flowering plants. Molliard (1916) has recently demonstrated that radish plants in pure culture are unable to increase the nitrogen content of the culture. The plants were grown in large tubes on nutrient solutions containing several different concentrations of ammonium chloride as a source of nitrogen, some cultures being supplied with glucose, and others being aerated with air charged with carbon dioxide in order to increase the photosynthetic activity. In no case did the total nitrogen of the crop and residual culture solution exceed that of the seed and original solution, even after six weeks' growth. The plants produced, however, were small, bearing three or four leaves in addition to the cotyledons, the green weight varying from 0.3 to 1.3 gr. Moreover, nitrate nitrogen was not supplied as a source of nitrogen in any case, a fact which may be of considerable importance in view of the results presented in this paper. It is possible that in the case of the higher plants also the proper culture conditions have not yet been realized, since in the case of four radish plants grown with the roots under aseptic conditions and the tops in free air, gains of 1.32 mg. nitrogen per plant were reported by Molliard, but he assumes that this nitrogen has come from the ammonia of the air.

The significance of the legume bacteria has recently been questioned by Beijerinck (1918) who argues that, with the exception of lupine and seradella, the number and weight of the tubercles is insignificant in comparison with the total weight of the plant. The isolated organisms fix nitrogen in pure culture only very slightly or not at all, and no fixation could be demonstrated with nodules placed in glass jars in which the changes of gases were measured. In the case of Robinia the number of tubercles is extremely small and fixation in them must be very intense, yet when tested they were found to be inactive or nearly so. He concludes that "the present accepted explanation of the behavior of legumes cannot be correct." The situation in the higher plants needs to be more carefully and thoroughly investigated.

Conclusions

1. Seven species of grass-green algae (Chlorophyceae) exhibited the ability to fix nitrogen when grown in pure cultures on mineral nutrient agar media containing either ammonium nitrate or calcium nitrate as a souce of nitrogen, and glucose. The nitrogen fixed was derived from the free (uncombined) nitrogen of the atmosphere. The amounts of fixation ranged

from 1 to 12.5 mg., representing increases in the total nitrogen content of the culture flasks of from 4 to 54 percent.

2. Five of the above mentioned species were grown on ammonium nitrate and calcium nitrate in the absence of glucose. Four of these showed slight increases in nitrogen over the original content of the medium; growth in the absence of glucose, however, was slight.

3. There was no fixation when urea, glycocoll, asparagine, or ammonium sulphate was supplied as a nitrogen source, either in the presence or in the absence of glucose or of mannite.

4. One species (no. 11) exhibited what is apparently a denitrification on media containing either ammonium nitrate or calcium nitrate as nitrogen sources, both in the absence of an organic carbon source and in the presence of mannite. The loss in nitrogen amounted to from 2.2 to 8.3 mg. per 100 grams culture medium.

ACKNOWLEDGMENTS

The writer wishes to express his sincere appreciation to Dr. J. R. Schramm, whose many helpful suggestions and constant interest have been invaluable aids in the completion of the experiments, to Dr. L. W. Sharp for the photographic work, and to other members of the department for kindly assistance in many ways.

DEPARTMENT OF BOTANY,
NEW YORK STATE COLLEGE OF AGRICULTURE,
ITHACA, NEW YORK

LITERATURE CITED

Beijerinck, M. W. The significance of the tubercle bacteria of the Papilionaceae for the host plant. Kon. Acad. Wetensch. Amsterdam (English ed.) **21**: 183–192. 1918.

Charpentier, P. G. Alimentation azotée d'une algue. Le *Cystococcus humicola.* Ann. Inst. Past. **17**: 321–334. 1903.

Duggar, B. M., and Davis, A. R. Studies in the physiology of the fungi. I. Nitrogen fixation. Ann. Mo. Bot. Gard. **3**: 413–437. 1916.

Fred, E. B. A physiological study of the legume bacteria. Ann. Rept. Va. Agric. Exp. Sta. 1911–12: 145–173. 1912.

Fred, E. B., and Graul, E. J. The effect of soluble nitrogenous salts on nodule formation. Jour. Amer. Soc. Agron. **8**: 316–328. 1916.

Freudenreich, E. von. Ueber stickstoffbindende Bakterien. Centralbl. Bakt. II. **10**: 514–522. 1903.

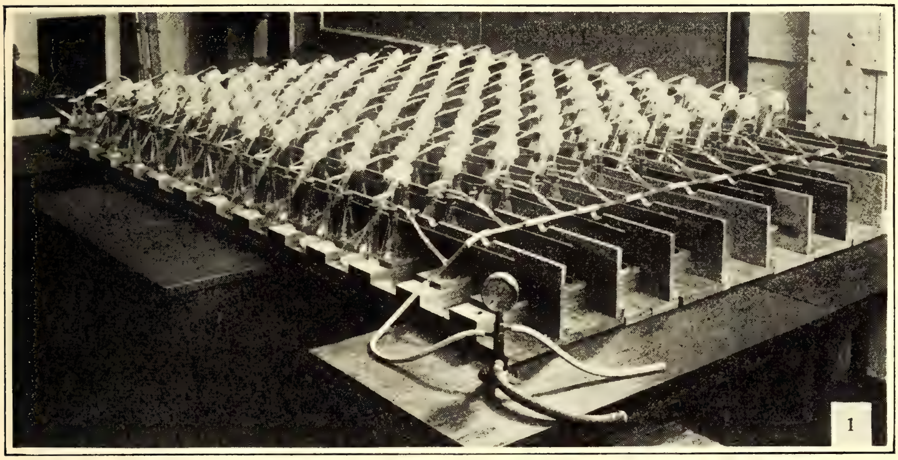
Gerlach, M., and Vogel, I. Weitere Versuche mit stickstoffbindenden Bakterien. Centralbl. Bakt. II. **9**: 817–821, 881–892. 1902.

Hills, T. L. Influence of nitrates on nitrogen-assimilating bacteria. Jour. Agric. Res. **12**: 183–230. 1918.

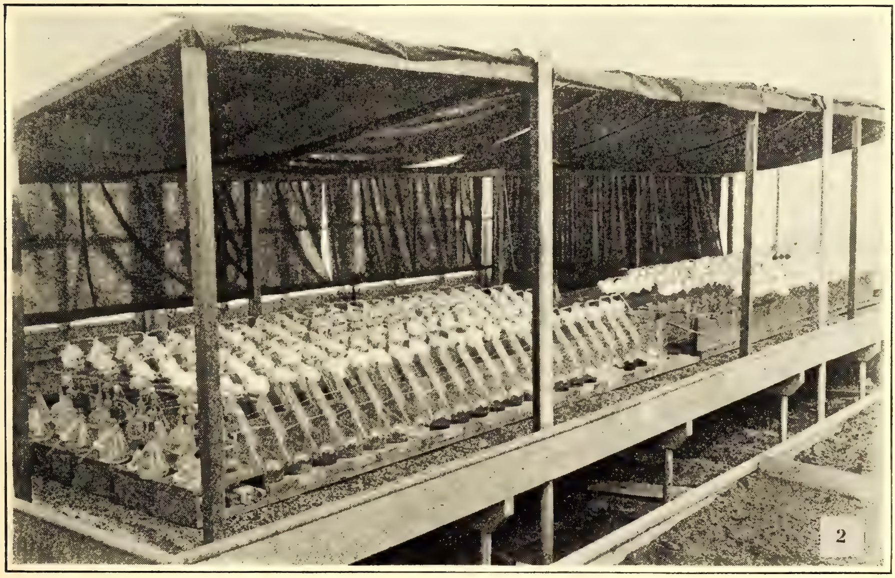
Hoffmann, C., and Hammer, B. W. Some factors concerned in the fixation of nitrogen by Azotobacter. Univ. Wis. Agric. Exp. Sta. Res. Bull. **12**: 155–172. 1910.

Kossowitsch, P. Untersuchungen über die Frage, ob die Algen freien Stickstoff fixiren. Bot. Zeit. **52**: 97–116. 1894.

Kruger, W., and Schneidewind, W. Sind niedere, Chlorophyllgrüne Algen imstande, den freien Stickstoff der Atmosphäre zu assimilieren und den Boden an Stickstoff zu bereichern? Landw. Jahrb. **29**: 771–804. 1900.



1



2

WANN: FIXATION OF FREE NITROGEN.

Krzemieniewska, H. Der Einfluss der Mineralbestandteile der Nährlösung auf die Entwicklung des Azotobakters. Bull. Intern. Acad. Sci. Cracovie, Cl. Math. et Nat. 1910: 376–413. 1910.

Krzemieniewski, S. Untersuchungen über *Azotobacter chroococcum* Beij. Bull. Intern. Acad. Sci. Cracovie, Cl. Math. et Nat. 1908: 929–1051. 1908.

Lipman, J. G. Experiments on the transformation and fixation of nitrogen by bacteria. New Jersey Agric. Exp. Sta. Rept. **24**: 217–285. 1903.

——. Report of soil chemist and bacteriologist. Azotobacter studies. N. J. Agric. Exp. Sta. Rept. **26**: 254–280. 1905.

Molliard, M. L'azote libre et les plantes supérieures. Rev. Gén. Bot. **28**: 225–250. 1916.

Remy, T., and Rosing, G. Über die biologische Reizwirkung natürlicher Humusstoffe. Centralbl. Bakt. II. **30**: 349–384. 1911.

Schramm, J. R. A contribution to our knowledge of the relation of certain species of grass-green algae to elementary nitrogen. Ann. Mo. Bot. Gard. **1**: 157–184. 1914 (*a*).

——. Some pure culture methods in the algae. Ann. Mo. Bot. Gard. **1**: 23–45. 1914 (*b*).

EXPLANATION OF PLATE I

FIG. 1. The complete apparatus of the 1917–18 experiment. The ten series are connected, on the right, by T-tubes, forming a single complete series for the purpose of aeration.

FIG. 2. The complete apparatus of the 1919 experiment, occupying a bench in the greenhouse. The series in the foreground is no. 6A (urea, with glucose), on which a very vigorous growth was produced by all species except no. 7 (*Protococcus* sp.); the fourth and fifth flasks from the right of the series were inoculated with this species. The check flasks (7th, 14th, and last from the left) stand out clearly because of the absence of growth in them.

VARIATIONS IN THE OSMOTIC CONCENTRATION OF THE GUARD CELLS DURING THE OPENING AND CLOSING OF STOMATA

R. G. Wiggans

(Received for publication July 12, 1920)

It has been repeatedly shown that the opening and closing of stomata are closely related to the turgor of the guard cells. The first critical study upon this point was made by von Mohl (1856). Following von Mohl, work has been done by Schwendener (1881), Kohl (1886), Francis Darwin (1898), Leitgeb (1886), and Lloyd (1908). These investigators state that the opening and closing of the stomata are due to variations in the turgor of the guard cells, and that the condition of the turgor of these cells depends, first, upon the abundance of water in the plant, and second, upon the amount of effective osmotic substances in the cells. It was supposed until recent researches that whenever a plant was not well supplied with water, or in other words in a slightly wilted condition, the stomata would always be closed. Many of the earlier investigators showed to their own satisfaction that whenever the plant begins to wilt the stomata invariably close. In more recent years, Laidlow and Knight (1916) and Lloyd (1912, 1913) have contradicted this statement after finding stomata still open when plants had become badly wilted.

The second factor upon which the turgor of the guard cells depends, that of the amount of effective osmotic substances, has not been given so much attention. The observations made by Lloyd (1908) and Darwin (1898) on the starch content of the guard cells as compared with the other tissues of the leaves, and those made later by Iljin (1914), are the most important. Lloyd found that the starch content in the guard cells is greatest in the early hours of the morning and gradually disappears until the time when the stomata are at their maximum width. After the stomata begin to close there is a gradual accumulation of starch until the maximum is again reached at some hour during the night. Lloyd also found that the fluctuation of the amount of starch within the plastids of the guard cells is accompanied by a complementary fluctuation of the oil content.

Iljin (1914) made similar observations on the starch content of the guard cells of several plants which he had under study. The important phase of his work, however, was the actual determination of the differences in osmotic concentration between the guard cells of the stomata and the cells of the other tissues of the leaf. For the purpose of determining the threshold concentration, he used concentrations of KNO_3 varying in strength from 0.125 to 3.00 normal. His experiments in general showed an extraordinarily high osmotic concentration for the guard cells when the stomata are open as compared with the osmotic concentration in the epidermis and leaf parenchyma. He found little or no difference between the osmotic concentration of the guard cells and that of the epidermis of the leaf parenchyma when the stomata are closed. The latter tissues always remained constant in their osmotic pressures. Table 1 will serve to illustrate the extreme differences in osmotic concentration which Iljin found between the guard cells and the parenchyma of the leaves.

TABLE 1

Name of Plant	Pressure in Atmospheres Guard Cells	Parenchyma
Senecio Doria	over 80	22.5
Senecio Doria	108	22.5
Centaurea orientalis	53.7	21.4
Centaurea orientalis	98	under 24
Iris pumila	90	" 24
Iris pumila	98	13
Eryngium campestra	98	19.1
Verbascum Lynchnitis	80.5	17.9
Veronica incana	90	45(?)

The concentration in all cases is expressed as pressure in atmospheres. The greatest difference in concentration between the guard cells and the parenchyma always occurred when the stomata were open to the greatest width. No determinations were made before the stomata were open in the morning, but the observations made at 8 a.m., 12 noon, 4 p.m., and 7:30 p.m. showed a marked decrease in the difference in osmotic concentration from 8 a.m. to 4 p.m., at which latter time the concentration was the same in all tissues.

Iljin does not explain his method of calculating osmotic concentration in atmospheres of pressure. However, the osmotic concentrations reported in this paper will be calculated according to the tables and formula given by Jones (1907). All of Jones' calculations of osmotic concentration are based on the depression of the freezing point and the concentration of the solution. He gives table 2 for calcium chloride.

TABLE 2

Concentration	Lowering of Freezing Point	Atmospheric Pressure
.102 mol.	.505	6.08
.153	.752	9.054
.204	1.012	12.184
.255	1.267	15.255
.306	1.537	18.505
.408	2.104	25.332
.510	2.681	32.279
.612	3.348	40.31
1.000	6.345	76.394

The third column is calculated from Jones' formula for osmotic concentration of solutions expressed in atmospheres of pressure. The formula is as follows:

$$\begin{array}{l}\text{Osmotic concentration} \\ \text{of a solution} \\ \text{expressed in at-} \\ \text{mospheres of} \\ \text{pressure}\end{array} = \begin{array}{l}\text{Freezing} \\ \text{point} \\ \text{depression of} \\ \text{that solution}\end{array} \times \frac{\begin{array}{l}22.4 \text{ osmotic concentration of a} \\ \text{molecular solution expressed} \\ \text{in atmospheres of pressure}\end{array}}{\begin{array}{l}1.86 \text{ freezing point depression of a} \\ \text{molecular solution}\end{array}}$$

In order to check the strength of the solutions used in the following experiments with those of Jones, the freezing-point depressions of three concentrations used were determined with the aid of the Beckman apparatus. These determinations checked very closely with those given by Jones for solutions of the same strength. Thus it was considered safe to use his results for all calculations.

The experiments to be reported in this paper were made with a view to securing more data on the specific problem of the variations in osmotic concentration (1) of the guard cells during the opening and closing of the stomata, and (2) of the guard cells as compared with the other cells of the epidermis.

METHODS

The same general method was used throughout the various tests herein recorded. A series of concentrations of $CaCl_2$[1] was made up varying in strength from 1.00 molecular to 0.06 molecular by dilutions from a stock solution of gram-molecular concentration. The respective concentrations were: 1.0; 0.8; 0.6; 0.5; 0.45; 0.40; 0.35; 0.30; 0.28; 0.26; 0.24; 0.22; 0.20; 0.18; 0.16; 0.14; 0.12; 0.10; 0.08; 0.06 molecular. This series was determined upon after some preliminary trials. In making tests for the threshold concentrations of the guard cells and also of the epidermal cells (both determinations being made at the same time) portions of the epidermis were removed from the lower surface of the leaf and placed on microscopic slides. Two drops of the solutions of varying concentrations were then placed on each section. These prepared slides were then placed under moist chambers for a period of five to ten minutes. At the end of this period the sections of epidermis were covered with cover glasses and examined microscopically for plasmolysis both of the guard cells and of the cells of the epidermis. In this way a complete series could be tested and examined in a comparatively short time. Flat petri dishes moistened with the various solutions were used as moist chambers for the slides to prevent evaporation and a consequent change in the concentration of the solution.

---

[1] $CaCl_2$ was used in these experiments in preference to KNO_3 because of the tendency of the latter to make the protoplasm more permeable.

MATERIAL

By preliminary tests, it was found difficult to determine when epidermal cells were plasmolyzed if they did not contain pigment. This made it necessary to choose plants possessing pigmented cells in the lower epidermis. It was fairly easy to determine when guard cells were plasmolyzed on account of the density of their protoplasm. The plants found best for making quick and accurate determinations were Cyclamen, Iresine, *Zebrina pendula*, and young beet. The leaves of all these plants possess pigmented cells in the lower epidermis. The epidermis may be separated rather freely from the leaf parenchyma. This fact increases the rapidity with which mounts and determinations can be made. The work was begun in the laboratory of the Department of Plant Physiology at Cornell University at the suggestion of Dr. Lewis Knudson, and carried to completion in the laboratories at the University of Missouri. The same methods were followed at both places, and as far as possible the same kinds of plants were used in the tests.

RESULTS OF TESTS

Zebrina pendula

The first tests recorded on *Zebrina pendula* were made on November 27. The results are shown graphically in figure 1. The first observation was made at 9:30 a.m. At 10:30 a.m. the highest osmotic concentration of the guard cells was observed. From this hour to sundown there was a gradual decrease. On the other hand, the threshold concentration of the epidermal

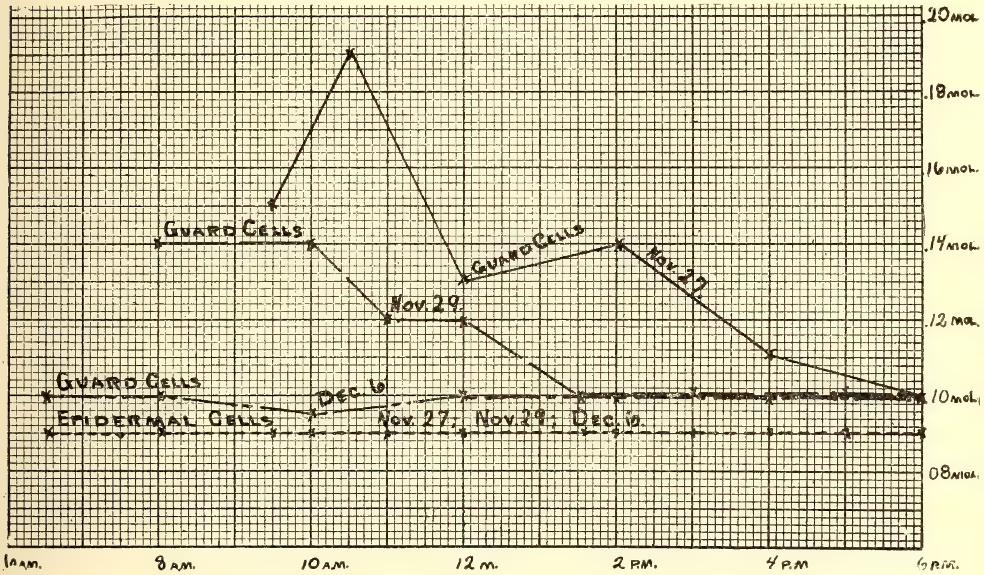


FIG. 1. *Zebrina pendula*. Observations made at Ithaca, N. Y.

cells remained the same throughout the day.[2] The greatest difference in osmotic concentration expressed in atmospheres was 5.97. It should be said in regard to the threshold concentration that it is difficult to determine exactly what concentration is the threshold, since all cells are not equally affected. The threshold concentrations in these tests were always considered the points at which the slightest plasmolysis could be detected, For these reasons there is bound to be some error in the determinations, but when differences in the concentration are large there is little difficulty in making observations. It is worthy of notice that the difference between the guard cells and the cells of the epidermis is very small as compared with the results secured by Iljin, although there is a rise with the opening of the stomata and a fall with their closing. The sun was visible 0.2 of the time from 8 to 9 a.m., and almost constantly from 9 a.m. to 12:30 p.m., after which there was no sunlight.

Further observations were made on *Zebrina pendula* on November 29, December 5, and December 6. The results of these observations are also shown graphically in figure 1, with the exception of those of December 5, when no differences were found. On November 29 the sun came out at 9:30 a.m. and was fairly bright the rest of the day. On December 5 there was practically no sunshine throughout the day. On December 6 the sun did not shine until 9:30 a.m., after which there was almost constant sunshine. On dark days there was very little if any opening of the stomata, although some stomata were always found open regardless of the time at which observations were made. On bright days there was a rather ready response of the stomata. They were usually open to their maximum by 10–11 a.m.

No observations were made on this plant at Columbia, Missouri, because no vigorous plants were available.

Cyclamen

The first recorded tests on cyclamen were made on November 29, when the sun shone most of the time after 9 a.m. The results are given in figure 2. There was no great difference between the osmotic concentration of the guard cells and that of the cells of the epidermis at any time. The greatest difference recorded was only a little over three atmospheres. The curve, however, indicates a rise in the osmotic concentration in the morning and a fall in the afternoon in the case of the guard cells (observations made at Ithaca).

The second series of observations was made on December 6, when the sunshine was rather constant after 9:30 a.m. These results are in almost perfect accord with those given for November 29 (observations made at Ithaca).

---

[2] The threshold concentration of the epidermal cells was found to be constant in all the experiments. Only slight variations in the readings occurred in every case. For this reason the threshold concentration of the epidermal cells is given as a straight line in the graphs.

Figure 3 shows the results of observations on cyclamen made on January 1 and January 2, 1918, at Columbia, Missouri. Here a new but entirely similar series of concentrations of $CaCl_2$ was used. The results recorded here were secured after several days of preliminary work with the new plants, apparatus, and solutions in order to make the error as small as possible.

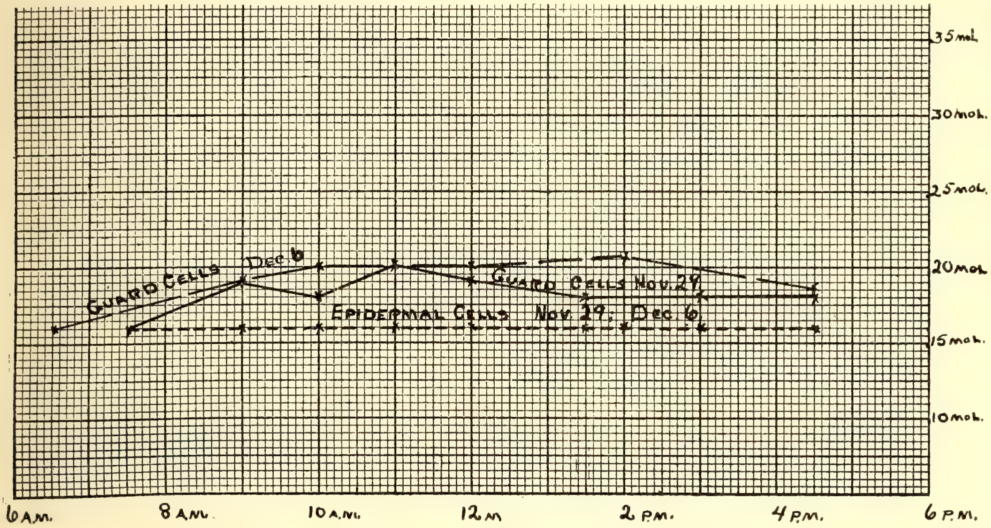


FIG. 2. Cyclamen. Observations made at Ithaca.

It is quickly seen that these results are very different from those secured at Ithaca. There is a rise in osmotic pressure in the morning and a fall in

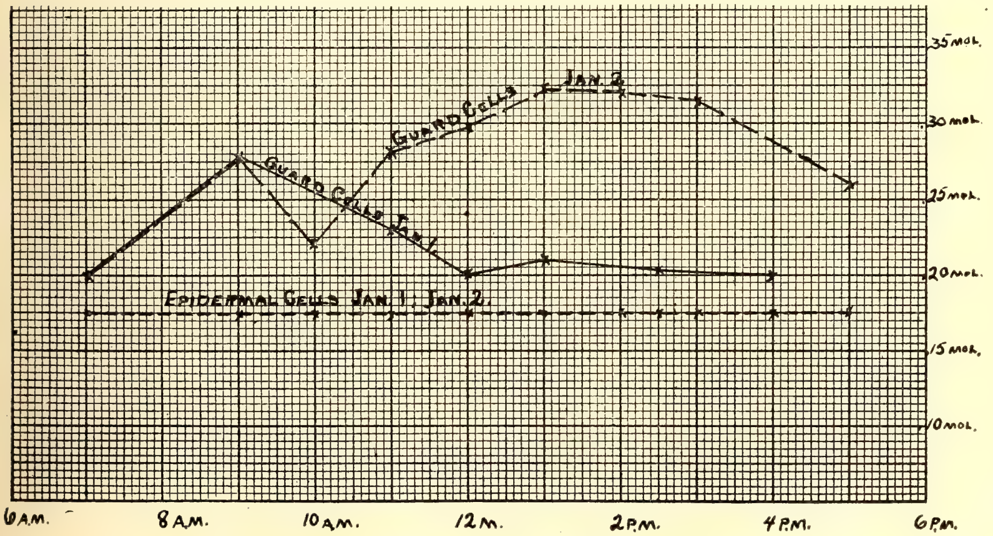


FIG. 3. Cyclamen. Observations made at Columbia, Mo.

the afternoon, as was indicated by the data secured at Ithaca, but the difference between the osmotic concentration of the guard cells and that of the cells of the epidermis is much greater. Expressed in atmospheres, the difference is 8.72 as compared with 3.00 at Ithaca.

The difference between the curves of January 1 and January 2 may be explained by the difference in the amount of sunshine. On January 1 the sun did not shine during the entire day, while on January 2 the sunshine was continuous in the afternoon although very limited in the forenoon.

Figure 4 shows a check series run with KNO_3 instead of $CaCl_2$. The same general curve is secured with these solutions as with solutions of $CaCl_2$; however, the molecular concentration in all cases was considerably higher. This is probably shown best by a comparison of the threshold concentrations of the epidermal cells in the two series.

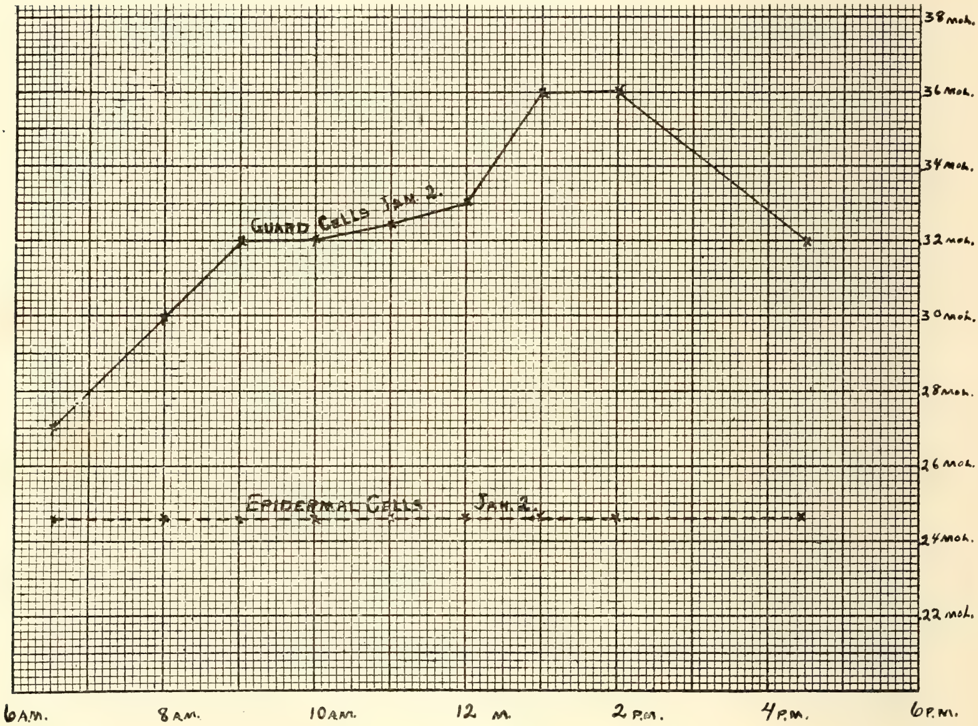


FIG. 4. Cyclamen. Observations made at Columbia.

Figure 5 shows the results of observations on cyclamen made at Columbia on January 3. The sun shone brightly until 1:45 p.m., after which there was no sunlight. These results accord with the results of January 2, with the exception that there was a greater difference between the osmotic concentrations of the epidermal and of the guard cells. Expressed in atmospheres, the greatest difference was 19.4.

The second curve shows the results of observations made on a check plant. With this exception all other tests at Columbia with cyclamen were made on the same plant. The perfect accord between these two plants, however, seems sufficient proof that the first plant was a normal one.

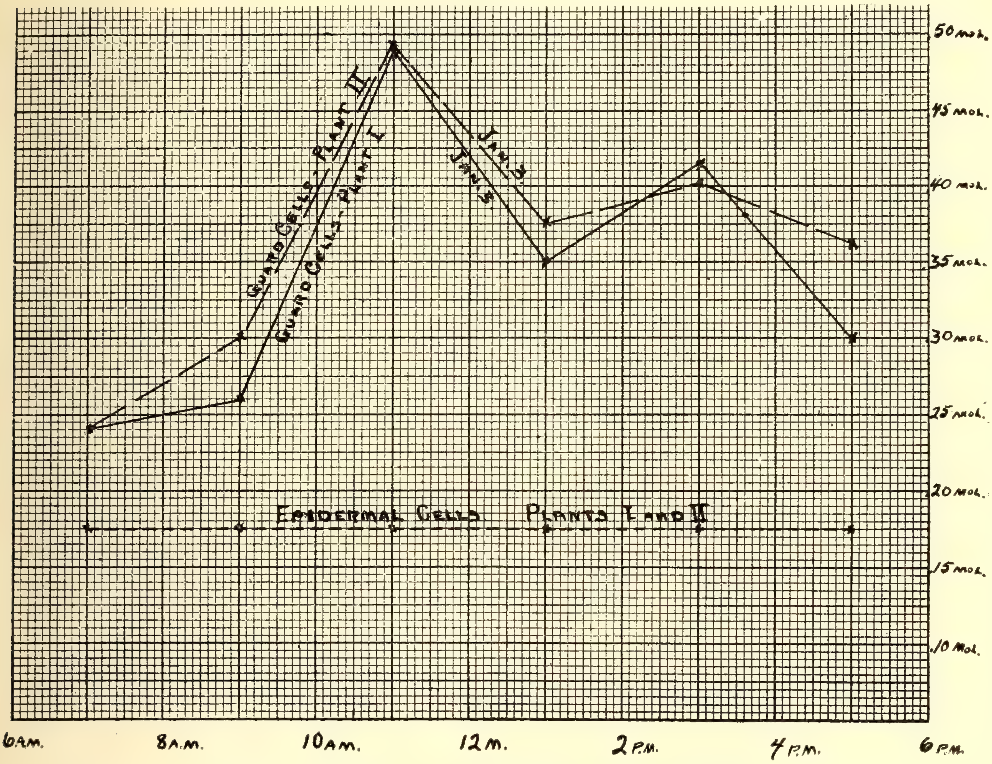


FIG. 5. Cyclamen. Observations made at Columbia.

Iresine

Figure 6 gives the results of observations on Iresine in a graphic manner. Curve (*a*) shows a rise in the osmotic concentration in the forenoon and a subsequent fall in the afternoon, with a maximum difference between the osmotic concentrations of the guard cells and the cells of the epidermis of 6.78 atmospheres. Curves (*b*) and (*c*) show a much greater rise in the forenoon and consequently a much greater fall in the afternoon than curve (*a*). Likewise the difference between the osmotic concentrations of guard cells and cells of epidermis is greater. The maximum difference amounted to 28 atmospheres.

Beet

Figure 7 shows the results of observations made on young beet plants at Columbia, Missouri, on January 4, when the sunshine was fairly constant throughout the day. The same general results were secured with this plant as with the other plants studied under similar environment. The greatest difference between the osmotic concentrations of the epidermal and the guard cells is 19 atmospheres' pressure.

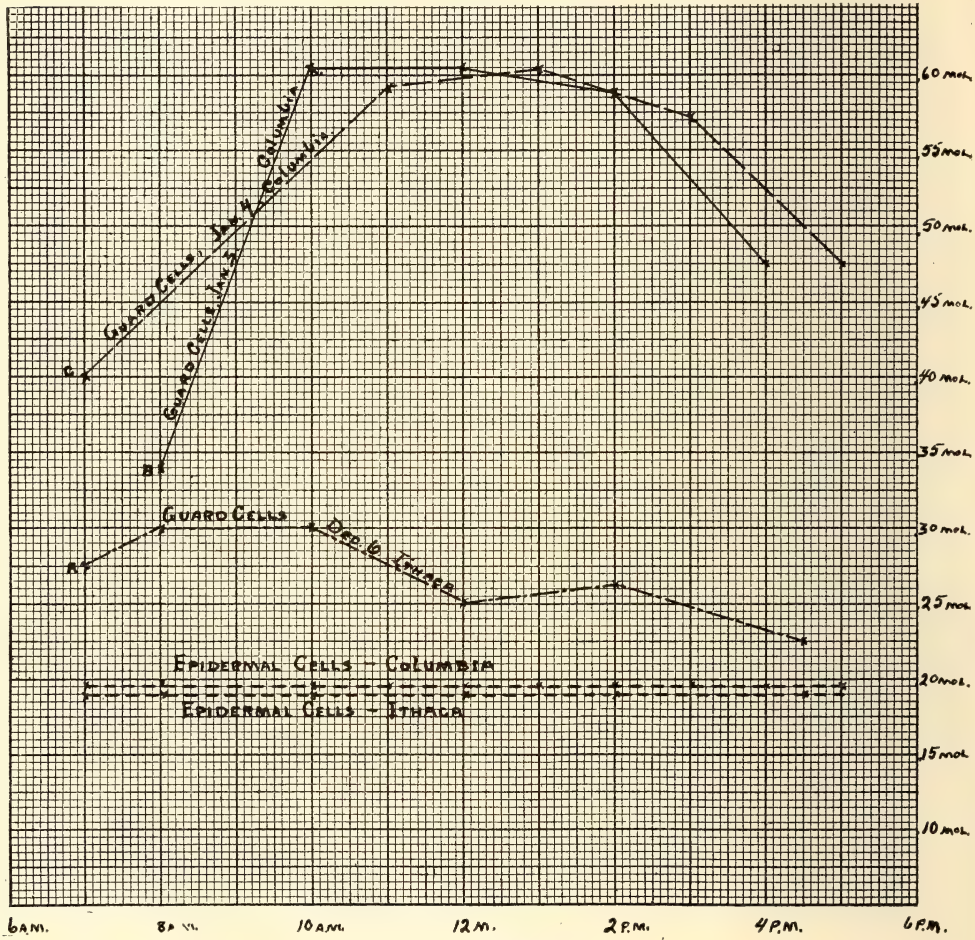


Fig. 6. Iresine.

Summary

The experimental data given in the foregoing charts on the whole do not show very great differences in osmotic concentrations between epidermal and guard cells. These differences may be summarized as in table 3.

These results are not in entire harmony with the results of Iljin, since the greatest difference here shown in osmotic concentration between guard and epidermal cells only approaches the smallest difference secured by Iljin.

The variations between the results at Ithaca and those at Columbia are difficult to explain, but may be due in part (1) to the individual plants employed in the experiments, (2) to the greater amount of sunshine at Columbia, and (3) to the greater temperature and humidity of the greenhouse in which the work was carried on at Columbia. There was never a time when the stomata were found completely closed at Columbia; most of the time they were found wide open. (On account of the equipment at Columbia it was impossible to determine the condition of the stomata at night.)

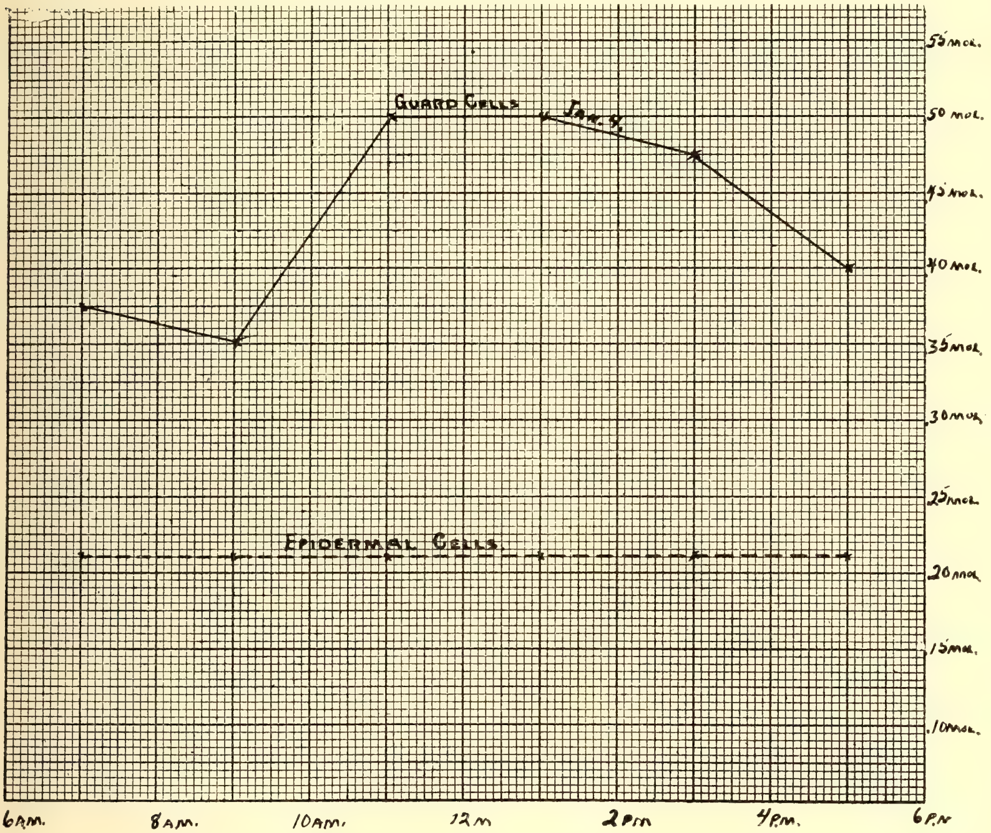


FIG. 7. Young beets. Observations made at Columbia.

TABLE 3

	Osmotic Concentration Expressed in Atmospheric Pressure			
	Ithaca, New York		Columbia, Missouri	
	Epidermis	Guard Cells	Epidermis	Guard Cells
Zebrina pendula	5.35	11.32		
Cyclamen	9.48	12.55	10.09	29.49
Iresine	11.33	18.11	11.63	39.52
Beets			12.55	31.50

The conclusions to be drawn from these experiments are:

(1) That there is a difference between the osmotic concentration of the guard cells of the stomata and that of the other epidermal cells when the stomata are open.

(2) That the osmotic concentration in the guard cells increases in the early hours of sunshine and decreases in the afternoon, approaching the osmotic concentration of the epidermal cells at nightfall.

(3) That there is very little if any change in the osmotic concentration of the epidermal cells during the day.

BIBLIOGRAPHY

Darwin, F. 1898. Observations on stomata. Phil. Trans. Roy. Soc. B**190**: 531–621.

Iljin, W. S. 1914. Die Regulierung der Spaltöffnungen mit der Veränderung des osmotischen Druckes. Beih. Bot. Centralbl. **32**: 15–36.

Jones, H. C. 1907. The elements of physical chemistry, pp. 236–256.

Kohl, F. C. 1886. Die Transpiration der Pflanzen und ihre Einwirkung auf die Ausbildung pflanzlicher Gewebe. Braunschweig.

Laidlow, C. G. P., and Knight, R. C. 1916. A description of the recording porometer and a note on stomatal behavior during wilting. Annals of Bot. **30**: 47–56.

Leitgeb, H. 1886. Beiträge zur Physiologie der Spaltöffnungsapparate. Mitteil. aus d. Bot. Inst. zu. Graz **1**: 125–183.

Lloyd, F. E. 1908. Physiology of stomata. Carnegie Institution of Washington, Publication 82, pp. 142.

——. 1912. The relation of transpiration and stomatal movements to water content of leaves of *Fouquieria splendens*. Plant World **15**: 1–14.

——. 1913. Leaf water and stomatal movement in Gossypium and a method of direct visual observation of stomata *in situ*. Bull. Torrey Bot. Club **40**: 1–26.

Mohl, H. von. 1856. Welche Ursachen bewirken die Erweiterung und Verengerung der Spaltöffnungen? Bot. Zeit. [cited by Iljin (1914)].

Schwendener, S. 1881. Über Bau und Mechanik der Spaltöffnungen. Monatsber. Berliner Akad. Wiss. [cited by Iljin (1914)].

THE LINNAEAN CONCEPT OF PEARL MILLET

Agnes Chase

(Received for publication July 19, 1920)

The commonly cultivated pearl millet has passed under more aliases, probably, than has any other grass. When the layman asks the botanist why he calls plants by such queer names the botanist tells him that it is for the sake of precision, that a given plant may bear a different common name in each country it inhabits, or in different parts of the same country, but that the Latin name is the same throughout the world. If the layman were acquainted with the Latin synonymy of pearl millet he could cite it to refute the botanist's defense. In the analysis of the Linnaean concept which follows, the common names, pearl millet and yellow foxtail, are used for the sake of precision instead of the Latin names. (The argument for Latin names is in the main, of course, valid, the exceptional case of pearl millet notwithstanding.)

Early in the last century pearl millet had almost as many names as there were floras. *Pennisetum typhoideum*, *Penicillaria spicata*, *Panicum spicatum*, and *Pennisetum alopecuroides* were the most popular. By the middle of the century the other names had mostly dropped out of use, giving place to *Pennisetum typhoideum*. In 1895, early in the period of modern nomenclatorial unrest which still agitates us, Dr. K. Schumann[1] "on the ground of priority" published for pearl millet the name *Pennisetum americanum*, based on "*Panicum americanum* L." In 1914 S. C. Stuntz took up the name *Pennisetum glaucum* (L.) R. Br., based on "*Panicum glaucum* L., based on a specimen from Ceylon." This is the name applied to pearl millet in recent American publications. Robert Brown applied the name *P. glaucum* to yellow foxtail, which was his understanding of Linnaeus's species. Mr. Stuntz published *Chaetochloa lutescens*, based on *Panicum lutescens* Weigel, for the foxtail.

Dr. Otto Stapf in a recent note on "*Setaria glauca* and *S. lutescens*," a copy of which was sent to Dr. A. S. Hitchcock before publication, holds that one should proceed with great caution before shuffling names which involve a grass of economic importance and also a well-known weed. With this opinion I heartily agree. All the names of pearl millet mentioned above are based upon Linnaean names. In order to arrive at a decision (necessitated in revising the North American species of Pennisetum), I have made an analysis of the Linnaean names involved in the problem.

---

[1] Page references are given in the bibliography.

1753. SPECIES PLANTARUM, PAGES 55, 56.

Panicum glaucum is composed of:

Panicum spica tereti, involucellis bifloris fasciculato-pilosis. *Fl. zeyl.* 44 [Pearl millet].

Gramen alopecuroides maderaspatanum, spica quasi geniculata molli. *Pluk. alm.* 177. *t.* 190. *f.* 6 [*Elytrophorus articulatus*].

β Gramen paniceum s. Panicum sylvestre, simplici spica. *Scheuch. gram.* 46 [*Chaetochloa viridis*].

γ Panicum spica simplici, aristis aggregatis flosculo subjectis. *Gron. virg.* 134 [Yellow foxtail].

Panicum indicum altissimum, spicis simplicibus mollibus in foliorum alis, pediculis longissimis insidentibus. *Tournef. inst.* 515 [Unidentifiable].

Habitat in Indiis.

Setae in spica longitudine flosculorum. Foliorum vaginae oris pilosae. Dum spica recens prodiit Flosculi in series dispositi observantur. [This description applies only to pearl millet.]

Dr. Stapf says it is very probable that the Hermann plants of the Flora Zeylanica were returned to their owner and were not at Linnaeus's hand when preparing the Species Plantarum, that Hermann's plants can not be accepted as types without further evidence. Dr. Stapf holds that such evidence is wanting in the case of *Panicum glaucum.* I should say the description ("Bristles the length of the flowers," and "In the young spike the flowers are seen to be disposed in series") supplies the evidence that Linnaeus had a plant of pearl millet at hand. The name *glaucum* itself applies well to the bluish head of pearl millet, not to the yellow head of the foxtail.

But, Dr. Stapf says, Linnaeus undoubtedly had Gronovius's plant (yellow foxtail) at hand. This specimen is written up as *Panicum glaucum* and numbered 2 by Linnaeus himself (the number of *P. glaucum* in the Species Plantarum). But may not this naming and numbering have been done after the publication of the name? The fact that the two references to Chaetochloa (3 and 4 above) are preceded by β and γ should also be considered. This method of indicating varieties was commonly used by Linnaeus in earlier works as well as in the Species Plantarum. It would seem that Linnaeus regarded the two species of Chaetochloa as varieties of *P. glaucum,* which itself was pearl millet.

If the case ended here (as, strictly following priority, it does) the evidence, because of the description, would, I think, point much more clearly to pearl millet. "Bristles of the spike as long as the flowers" agrees with pearl millet; in yellow foxtail they are much longer. "In the young spike the flowers are seen to be disposed in series" points undoubtedly to the crowded fascicles of pearl millet that appear to run obliquely like the cells of a honeycomb. But in Systema Nature ed. 10. (**2**: 870. 1759) "seminibus undulato-rugosis. Sp. Pl. n. 2. γ." is added to the diagnosis for *P. glaucum,* which otherwise is taken verbatim from the Flora Zeylanica reference in the first edition and applies to pearl millet. "Seminibus undulato-rugosis" applies only to the Gronovian plant, yellow foxtail. The reference to no. 2 γ would indicate that Linnaeus wishes to attach the name *glaucum* to the Gronovian plant. The variety β of the first edition is now named *P. viride.* The diagnosis is an exact repetition of that for *glaucum* just above except for the last phrase, which is "seminibus nervosis." The "fasciculato pilosis" does not apply to this species of Chaetochloa any more than it does to the other.

Paralleling this evolution of *P. glaucum* in Linnaeus's mind is that of *P. alopecuroides*, the first species of Panicum in the Species Plantarum (page 55). "Habitat in China" is given as the source of that species. Concerning the plant in the Linnaean Herbarium, Dr. Stapf writes:

> "The diagnosis and the description fit the plant very well. It is the same plant which R. Brown described subsequently as *Pennisetum compressum.* The specimen is numbered 1—the number of *Panicum alopecuroideum* in Species Plantarum, ed. 1.—by Linnaeus, and the country given by him is 'Chin.' The name *alopecuroideum* is not in his handwriting. There is, however, another sheet, written up by him '*alopecuroideum,*' but this is not numbered, nor is there anything to show where he had it from; this is a starved specimen of *Pennisetum spicatum* = *P. americanum* f. (26) *sieberianum* Leeke" [one of the forms of pearl millet].

Besides the diagnosis and unusually good description Linnaeus cites a figure of Plukenet which, according to Trinius and Stapf (and to all appearances), represents *Perotis latifolia.* In the Systema (**2**: 870) "basi ciliatis" is inserted in the diagnosis following "involucris setaceis." Bristles with ciliate base are found not in the Chinese species but in pearl millet.

A third name involved is *P. americanum*, the third species of Panicum in the Species Plantarum (page 56). This is composed of

Panicum spica simplici aequali, pedunculis bifloris. *Roy. lugdb.* 54. [Unidentifiable by the writer; may be pearl millet.]
Panicum indicum, spica obtusa caerulea. *Bauh. pin.* 7. *theatr.* 522. [The "theatr." referred to is the illustrated Theatri botanici, 1658. The figure is copied, by tracing evidently, since it is reversed, from that in Clusius (see below) illustrating *Panicum americanum.*]
Panicum americanum *Clus. hist.* 2. *p.* 215. [The figure referred to is a branching plant with thick heads, about half as broad as long, in the axils of the upper leaves. It can not by any stretch of imagination be taken for pearl millet. The description suggests a large form of common millet, *Chaetochloa italica*].
"*Habitat in* America."

Since there is no description, we may assume that Linnaeus was naming a species he did not know, that is, he was giving a name to certain references in books. Since he appropriates the Clusian name that may be taken as the basis of his name. Clusius's species being unidentifiable, the name may be rejected. A figure on page 216 of Clusius's work entitled "Panicum Americ. sesquipedalis spica" is unmistakably pearl millet. But it is not this figure that Linnaeus cites, nor the Clusian description of it, differentiating it from his *Panicum americanum*. In 1759 (Syst. Nat. ed. 10. **2**: 870) the phrase name cited in the Species Plantarum from "Roy. lugdb. 54," is given, but the reference to Royen is omitted. In the second edition of Species Plantarum (1762, p. 82) *Panicum americanum* is omitted. The phrase names cited under it in the first edition are now placed under *Holcus spicatus* (p. 1484). The name was not used for a species in any subsequent work of Linnaeus, nor elsewhere until taken up by Schumann in 1895, and transferred to Pennisetum. Schumann says the plant was sent to Linnaeus from America, but there is no evidence that he ever had an actual specimen that he called *Panicum americanum*.

Following "*alopecuroides* 1" with its altered diagnosis in the Systema is "*cynosuroid.* A. P[anicum] spica tereti involucellis unifloris, raiis pilosis," nothing more. The peduncles of the fascicles in the Chinese species are pilose. Having applied "*alopecuroides*" to pearl millet did Linnaeus mean to call the Chinese plant "*cynosuroides*"? If so he changed his mind, for he never uses *P. cynosuroides* again.

In the second volume of the Systema (1759, page 1305) another factor enters into the problem. Panicum is placed under Triandria Digynia. Under Polygamia Monoecia, in the genus Holcus (containing two cultivated sorghums, Johnson grass, and six other species not congeneric with the sorghums), is published *Holcus spicatus* "glumis bifloris muticis, floribus geminis penicillo involucratis, spica ovato-oblonga. *Pluk.* t. 32. f. 4." The diagnosis is original and applies well enough to pearl millet. Plukenet's figure is also very probably pearl millet.

So far we have: (1) *alopecuroides* altered to fit pearl millet (China is never again mentioned in connection with this name; (2) *cynosuroides* (probably a species of Pennisetum), a name not to appear again; (3) *glaucum*, the diagnosis altered and applied to var. γ of the Species Plantarum, but with part of the original diagnosis (applying to pearl millet but not to yellow foxtail) remaining; (4) *Holcus spicatus*, the diagnosis applying fairly well to pearl millet and the figure cited almost certainly meant for that species.

1762. Species Plantarum ed. 2: 82, 83.

Panicum alopecuroideum is here composed of: (1) The altered diagnosis from the Systema [the bristles ciliate at base applying to pearl millet]. (2) The reference to Plukenet's figure of *Perotis latifolia* queried. (3) "Gramen indicum alopecuroides holosericum majus, spica longa pappescente. *Pluk. alm.* 177. *t.* 92. *f.* 5." [The figure is unidentifiable. I took it for *Pennisetum polystachyum* (L.) Schult. of India, but Dr. Stapf writes that a sample of the Plukenet original in the Morison Herbarium at Oxford is *Melica ciliata*. With that species in mind I can see that it looks more like that than it does like *P. polystachyum*.] (4) "*Habitat in* Jamaica." [This habitat is unaccountable.] (5) The description, unaltered, from the first edition [applying to the Chinese plant].

Panicum glaucum is here composed of: (1) The diagnosis given in the Systema (part of it applying to pearl millet and part to yellow foxtail), followed by "*Fl. zey.* 44" (pearl millet only). (2) The Gronovian diagnosis and reference, without "γ" (applying to yellow foxtail). (3) "*Habitat in* Indiis & Italia" (notwithstanding the reference to Gronovius, Flora virginica). (4) The description verbatim from *P. glaucum* in the first edition (the statement that the bristles are the length of the flowers, and the observation of the flowers disposed in series, applying conclusively to pearl millet), with the addition "Semina striis undulatis notata" (applying to the fruit of yellow foxtail and not to that of pearl millet) as in the Systema.

Holcus spicatus (page 1483) is composed of:

Holcus glumis bifloris muticis, floribus geminis penicillo involucratis, spica ovato-oblonga [the diagnosis from the Systema, 1759].

Panicum spica simplici aequali, pedunculis bifloris. *Roy. lugdb.* 54 [Unidentifiable by the writer, may well be pearl millet].

Panicum indicum, spica obtusa caerulea. *Bauh. pin.* 7. *theatr.* 522. [The second citation under *P. americanum* in the first edition of Species Plantarum. The "theatr." referred to is the illustrated Theatri botanici, 1658. The figure is copied by tracing from that in Clusius for *P. americanum*, and does not represent pearl millet. The long description says among other things that the culm near the base is of an elegant blue and shining purple and the pith spongy, characters that suggest sorghum. But the further description of the culm as dividing into branches does not apply to sorghum. (Was it perhaps the illustration that was described?) The spike is said to be sometimes a finger long and sometimes only an inch, and to resemble "Frumenti Turcidi" (maize). It is said to be from the Indies and also from Peru, to be grown in gardens in Belgium, rarely in Germany, from seed sent from Spain. Altogether it reads like a compound of half-remembered plants of sorghum, common millet, and maize. Such a figure and description at any rate can not reasonably be taken as a basis for a name.]

Panicum americanum. *Clus. hist.* 2. *p.* 215. [See note on *P. americanum* above.]

Gramen alopecuroides indicum maximum. *Raj. hist.* 1908. [Ray's description applies very well to pearl millet.]

Gramen paniceum sylvestre maximum indiae orient. *Pluk. alm.* 164 [error for 174] *t.* 32. *f.* 4. [The figure, which was referred to under *H. spicatus* in the Systema, may well be pearl millet.]

Habitat in India.

Culmus bipedalis, crassitie pennae cygneae, tectus vaginis foliorum hispidis ut ipse culmus. Folia saepius 10, latitudine digiti hispida. Spica crassissima pedicellis brevissimis apice fasciculo setarum, intra quem Flores 2, sessiles. Calyx bivalvis, membranaceus, biflorus. Petalo exteriore hermaphroditi mucronato; masculi obtuso. Stylus floribus longior, lanatus, laeviter apicae bifidus. Antherae oblongae. [A hispid culm two feet tall, and as thick as a swan's quill, covered with hispid sheaths and with hispid blades, is certainly not pearl millet, but the rest of the description might apply to it, or, somewhat better, to common millet. Did Linnaeus possibly have a sterile plant of

Panicum miliaceum and a head of pearl millet or of common millet when he wrote the description?]

Summing up the problem as it stands after the publication of the second edition of the Species Plantarum, we have:

1. *P. alopecuroides*: the diagnosis applying to pearl millet, the description to the Chinese species, the citations from Plukenet and the habitat applying to neither.

P. cynosuroides and *P. americanum* are omitted.

2. *P. glaucum*: both diagnosis and description are composites of the characters of pearl millet and yellow foxtail; the citation from Gronovius refers to the foxtail.

3. *Holcus spicatus*: the diagnosis with a minor exception applies to pearl millet; the description applies partly to *Panicum miliaceum* (?) and partly to pearl millet (?) Three of the citations refer to pearl millet and two to an unidentifiable figure.

1767. SYSTEMA NATURAE ED. 12: 86–87, 669.

Panicum alopecuroides is exactly that of the tenth edition of the Systema [altered from Species Plantarum to fit pearl millet].

Panicum glaucum: the diagnosis is that of the tenth edition [applying to pearl millet], the reference to "Sp. Pl." omitted, and "Pedunculus valde sulcatus" applying to yellow foxtail added.

Holcus spicatus: the diagnosis is that of the tenth edition, applying fairly well to pearl millet. The reference to Plukenet is omitted.

1771. MANTISSA PLANTARUM 2: 322.

"Panicum alopecuroid. Excludatur et reformatum restituatur sequentibus." The diagnosis now reads: "spica tereti, involucellis setaceis fasciculatis bifloris, pedunculis villosis" [applying well to pearl millet]. The second Plukenet reference (that to the unidentifiable *pl.* 92. *f.* 5) is cited, "*Habitat in* India *orientali.*" The description given in the first and second editions of the Species Plantarum is dropped and a new one inserted: "Statura Panici italici. Culmi et totum villosum. Folia latitudine digiti transversi, utrinque pilosa, etiam vaginis. Spica magnitudine digiti ex Involucellis multiseto-fasciculatis, villosis, scabris, bifloris, pedicellatis, longitudine flosculorum. Florum valvula accessoria longitudine reliquarum." [The statement that the sheaths and both sides of the blades are pilose does not apply to pearl millet, nor does that of the accessory valve as long as the rest, whether either of the glumes or the sterile lemma is meant. If Linnaeus had a ripe spikelet of pearl millet with the globose grain forcing apart the lemma and palea, he might have meant that the lemma, palea, and grain are of equal length, which would be correct.]

Panicum glaucum and *Holcus spicatus* are not given in the Mantissa, being in their author's mind, doubtless, in no need of correction.

From the foregoing it seems quite probable that, as in the case of subsequent authors, Linnaeus sometimes had very vague or confused "concepts" and that, like many another busy author, he "revised" his books with a pair of scissors and a paste cup. May it not be possible, even, that some of the students whose botanical papers form Linnaeus's numerous Dissertationes academicae, wielded the scissors and paste brush for him? At any rate the revisions do not show careful reconsideration. Should subsequent changes of diagnoses, that in each case serve to blend further the diverse elements, outweigh the original almost clear application of the name *Panicum glaucum* to pearl millet?

So much for Linnaeus's concept; now to take a rapid survey of the species as treated by subsequent botanists. Pearl millet was not generally confused with other species, but there were diverse views as to its proper generic position, all realizing that it did not belong to either Panicum or Holcus. In Murray's revision of Linnaeus's Systema Vegetabilium, 1774, he placed *Panicum alopecuroides* (itself uncertain as shown above, but applied to pearl millet by Murray) in Alopecurus (*A. indica*). Cavanilles in 1802 placed it in Cenchrus. In 1805 L. Richard (in Persoon's Synopsis Plantarum) established the genus Pennisetum for this and allied species, renaming pearl millet *P. typhoideum*, possibly, because of the confusion in the Linnaean names, wishing to reject them all. In 1809 Willdenow, apparently unacquainted with the recently published Pennisetum, proposed Penicillaria for pearl millet. Running through the more important subsequent botanical works containing the species we find:

Pennisetum typhoideum used by Persoon, 1805; Sprengel, 1825; Trinius, 1826, 1834; Steudel, 1854; Hooker, 1896; Stapf, 1898; Watt, 1892, 1908; Trimen, 1900; Battandier and Trabut, 1902; Cooke, 1908.

Penicillaria spicata used by Willdenow, 1809; Roemer and Schultes, 1817; Link, 1821; Kunth, 1823; Nash, 1903.

Pennisetum americanum used by Schumann, 1895; Leeke, 1907; Hitchcock, 1908.

Pennisetum spicatum used by Körnicke, 1885; Beal, 1887.

Pennisetum glaucum used by Stuntz, 1914; Hitchcock, 1920. (Brown used this name for yellow foxtail.)

Panicum spicatum used by Roxburgh, 1820.

Cenchrus spicatus used by Poiret in Lamarck's Encyclopedia, 1816.

It will be seen that *Pennisetum typhoideum* has been the favorite in recent years but has not had a majority.

In 1916 Drs. Schinz and Thellung discussed the case. They state that *Panicum glaucum* L., 1753, is a composite (Sammelart) of three different species. [If the citations given as belonging to the species itself as well as β and γ are included, it contains six.] The authors further state that in 1759 (in the Systema) Linnaeus himself restricted the name to his earlier var. γ. But in the Systema we have "P. spica tereti, involucellis bifloris fasciculata-pilosis (applying to pearl millet and not to yellow foxtail), seminibus undulato-rugosis. Sp. pl. n. 2. γ" (applying to yellow foxtail and not to pearl millet). Instead of "restricting" the name to either species Linnaeus adjusts it to both. In the foxtail the spikelets are solitary in each fascicle and the fascicles are not pilose. In pearl millet the spikelets are usually two to the fascicle and the fascicles are pilose. In the second edition of the Species Plantarum, which affords greater space, Linnaeus again cites "Fl. zey. 44" (pearl millet only) and gives the original description of pearl millet, adding to it that of the fruit of yellow foxtail.

Now "in the interests of stable nomenclature" what can be done to bring order out of this confusion?

My own judgment would be that:

1. *Panicum alopecuroides* be restricted to that of the Species Plantarum, 1753, the citations discarded, leaving the Chinese specimen as the type (= *Pennisetum alopecuroides* (L.) Spreng., but not as Sprengel applied the name).
2. *Panicum glaucum* be restricted to its first diagnosis and description, applying clearly to pearl millet, and that the citations, including the varieties β and γ, be discarded. The name *glaucum* itself applies to the bluish tinge of the spike of pearl millet (= *Pennisetum glaucum* (L.) R. Br., but not as Brown applied the name).
3. *Panicum americanum* be rejected, since it was based on two unidentifiable figures, one evidently drawn from a tracing of the other.
4. *Panicum cynosuroides* be rejected. This seems to have been used but once, by Scopoli in 1778, and applied to *Chaetochloa viridis*.
5. *Holcus spicatus* be restricted to its first diagnosis in 1759, becoming a synonym of *Pennisetum glaucum.*

The object in publishing this lengthy analysis is primarily to make available for the use of others what has cost much time and study. But a second reason is that it furnishes a good example of the "Linnaean concept of species," to which botanists who are not systematists sometimes bid us return. This is not to find fault with Linnaeus, it is only to show that he was human and fallible like the rest of us. His concept of species was not "broader" than ours, as is commonly supposed by those who have not used his books. He described very closely allied forms, such as *Bromus purgans* and *B. ciliatus,* or even "split" a single species, as when he described *Andropogon divaricatum* and *A. alopecuroides* for the commonest Erianthus of our eastern states (*E. divaricatus* (L.) Hitchc.). When Linnaeus had a plant in hand his descriptions are often vivid impressionist pictures. That of *Panicum dichotomum* "like a little tree, simple below and branching above," recalls the autumnal phase of the plant instantly *to one who knows it.* But in the majority of his species there is no description but the brief diagnosis following the name, which is often inadequate for identification.

From the case here presented the botanist who is not a systematist may also see some of the reasons why we have codes of nomenclature and why with all our codes we have not as yet attained stability.

DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.

BIBLIOGRAPHY

Battandier and Trabut. Fl. Algerie and Tunis 359. 1902.

Bauhin, C. Pinax 27. 1623.
Theatri botanici 522. 1658.

Beal. Grasses N. Amer. **1**: 187. 1887.

Brown, R., Prodr. Fl. Nov. Holl. **1**: 195. 1810.

Cavanilles. Descript. 304. 1802.

Clusius. Rar. Pl. Hist. **2**: 215. 1601.

Cooke. Fl. Bombay 917. 1908.

Gronovius. Fl. Virg. 134. 1762. (I am unable to verify this reference.)

Hitchcock, A. S. Contr. U. S. Nat. Herb. **12**: 132. 1908; U. S. Dept. Agr. Bull. **772**: 246. 1920; Contr. U. S. Nat. Herb. **22**: 165. 1920.

Hooker, f. Fl. British India **7**: 82. 1896.

Körnicke and Werner. Handb. Getreid. **1**: 284. 1885.

Kunth. Enum. Pl. **1**: 165. 1823.

Leeke. Zeitschr. Naturwiss. **79**: 52–55; 67. 1907.

Link. Enum. Pl. **1**: 78. 1821.

Linnaeus. Flora Zeylanica 18. 1747.
Mantissa, vol. 2. 1771 (page references are given in the text).
Sp. Pl. 1753. Ed. 2, vol. 1. 1762; vol. 2. 1763.
Systema Naturae, ed. 10. 1759; ed. 12. 1767.

Nash. In Small. Fl. Southeast. U. S. 110. 1903.

Murray. Syst. 92. 1774.

Plukenet. Almagest. 174, 177. 1696. Phytograph. *pl.* 32, *f.* 4; *pl.* 92, *f.* 5; *pl.* 190, *f.* 6. 1691.

Poiret. In Lamarck. Encycl. Suppl. **4**: 351. 1816.

Ray. Historia Plant. 1908. 1688.

Richard, L. In Persoon. Syn. Pl. **1**: 72. 1805.

Roemer and Schultes. Syst. Veg. **2**: 498. 1817.

Roxburgh. Fl. Indica **1**: 286. 1820.

Royen. Fl. Leyden Prod. 54. 1740.

Scheuchzer. Agrost. (sive Gram.) 46. 1719.

Schinz and Thellung. Vierteljahrschr. Naturf. Ges. Zürich **61**: 419–421. 1916.

Schultes. Mant. 282. 1824.

Schumann, K. In Engler. Pflanzenw. Ost-Afr. 5^{B}: 51. 1895.

Scopoli. Fl. Carniola, ed. 2, **1**: 50. 1778.

Sprengel. Syst. Veg. **1**: 303. 1825.

Stapf. In Thiselton-Dyer. Fl. Cap. **7**: 431. 1898.

Steudel. Syn. Pl. Glum. **1**: 108. 1854.

Stuntz. U. S. Dept. Agr. Bur. Pl. Ind. Inventory Seeds **31**: 84, 86. 1914.

Tournefort. Institutiones **1**: 515. 1719.

Trimen. Flora Ceylon 170. 1900.

Trinius. Clav. Agrost. 206, 353. 1822.
Gram. Pan. 70. 1826.
Mem. Acad. St. Pétersb. Sci. Nat. VI. **1**: 184. 1834.

Willdenow. Enum. Pl. 1037. 1809.

THE EFFECT OF CLOUDINESS ON THE OXYGEN CONTENT OF WATER AND ITS SIGNIFICANCE IN CRANBERRY CULTURE

H. F. Bergman

(Received for publication July 22, 1920)

In the culture of cranberries it is often necessary to flood the bogs as a protection against frost and certain insects. The length of the flooding period may vary from a few hours to several days. To ascertain the relation between the oxygen content of waters and injuries which had occurred to the vines as a result of flooding, an investigation was begun in 1918. This work was carried on during the seasons of 1918 and 1919 in the cranberry regions of Massachusetts and Wisconsin.

One of the first things that became apparent as a result of the study of flooding water of cranberry bogs was the extent of variation of the oxygen and carbon dioxide content of water from different sources and the correlation of these variations with weather and other conditions. The oxygen and carbon dioxide content of exposed waters under certain conditions varies considerably as between day and night. The extent of this variation is affected by the amount of sunlight during the day as determined by the presence or absence of clouds. The velocity and direction of the wind and the temperature of the water also affect the gas content of flooding waters but are less important than light intensity.

The effect of cloudiness on the oxygen and carbon dioxide content of water is indirect, resulting from the action of light on submerged vegetation. When plants are submerged, oxygen and carbon dioxide during respiration or photosynthesis are absorbed from the water or given off into it in solution. Accordingly, the oxygen content of the water is increased during the day in proportion to the amount of vegetation present and to the light intensity, while the carbon dioxide content decreases under the same conditions.

The presence of oxidizable organic matter in the water or in the substratum tends to reduce the oxygen and to increase the carbon dioxide content of the water. This effect has been observed in the marshes, used by many Wisconsin cranberry growers for reservoirs, and in the water of cedar swamp reservoirs in Massachusetts. On a clear day the amount of oxygen produced by photosynthesis is much in excess of that used up in respiration or by the oxidation of organic matter. For this reason, when vegetation is present, an accumulation of oxygen occurs. In cloudy weather the accumulation is less. The oxygen content may even decline in densely cloudy weather. A diurnal variation is thus to be expected in ponds with vegetation and decaying organic matter, the amplitude of variation depending on the relative amount of vegetation and of organic matter and on the intensity of the light.

In regard to diurnal variation in the oxygen content of lake water Birge and Juday (1, p. 53) state that:

> "Some European observers have noted marked diurnal changes in the amount of dissolved oxygen in small, shallow bodies of water, especially fish ponds, and attention has been called to such changes in the upper water of Lake Mendota in September, 1908 (p. 43). In Mendota, the excess oxygen stratum comprised only the upper two or three meters which were readily disturbed by wind and by convection currents. Thus there was a pretty thorough mixture of this upper water at night so that the oxygen became uniformly disturbed in this stratum. As a consequence, there was an appreciable decrease in the quantity of oxygen in the layer having the largest excess, that is at a depth of 1.5 m., and on the succeeding day the amount of oxygen would again decrease at this depth, thus producing a diurnal variation of 1 cc. to 1.5 cc. per liter of water. But where the excess oxygen has been found in the thermocline region, no appreciable diurnal variations have been noted. That is, the difference between day and night observations did not prove to be any greater than the differences between two sets of day observations, one of which was made immediately after the other."

Birge and Juday were dealing mostly with large, deep bodies of water containing relatively little vegetation and with bottoms free of organic deposits. Under such conditions the oxygen content of the water depends chiefly upon the temperature of the water. According to Pettersson and Sonden (5, p. 1443), a liter of water at 0° C. and 760 mm. pressure is capable of absorbing from the atmosphere 10.01 cc. of oxygen. Roscoe and Lunt (6) state that under these conditions water requires only 9.7 cc. of oxygen for saturation. The oxygen capacity decreases with a rise of temperature. Thus Roscoe and Lunt give 6.28 cc. as the amount of oxygen required for saturation of water at 20° C. and 5.76 cc. at 25° under 760 mm. pressure.

The latitude of variation in the carbon dioxide and oxygen content of waters in the cranberry region of Massachusetts may be shown by a few typical examples presented in table 1.

Spectacle Pond shows the least variation in carbon dioxide and oxygen content from day to day and between clear and cloudy weather. The carbon dioxide content ranges from 0.1 to 0.7 cc. per liter and the oxygen content from 5.4 to 6.5 cc. per liter. In Cedar Pond the range is greater, the carbon dioxide content varying from 0.4 to 2.8 cc. per liter depending on weather conditions and place of sampling. The oxygen content of Cedar Pond water varies from 4.8 to 6.0 cc. per liter. The greatest variation occurs in the water of the State Bog ditch, the carbon dioxide content ranging from 2.0 to 10.1 cc. per liter. The oxygen content similarly has a wide range, from 1.8 to 5.0 cc. per liter. The same relation obtained in 1919, as shown in figure 1.

Although there is some variation in temperature on the different days and hours at which these readings were made, this variation is not sufficient to account for the difference in gas content. These samples, with the exception of those taken from Spectacle Pond July 30 and from the State Bog August 10, were all taken in clear weather in shallow water near shore. The bottom of Spectacle Pond is clean white sand and the water very clear. In Cedar Pond the water is clear but the bottom is muck, consisting of vegetable matter most of which is well decomposed. The water in the ditches of the State Bog is discolored, of a very dilute coffee color as viewed by transmitted light, and the bottom is muck as in Cedar Pond. Spectacle Pond, moreover, has very little vegetation, which accounts for the almost constant oxygen and carbon dioxide content from day to day or between day and night periods. On the other hand, Cedar Pond is well filled with water lilies and other vegetation which modifies the oxygen and carbon dioxide content of the water materially through respiratory and photosynthetic activities. In the State Bog ditches a more or less abundant growth of algae is found which brings about the same result. Accordingly, both in Cedar Pond and in the ditches of the State Bog a wide variation in carbon dioxide and oxygen content of the water is observed between day and night or between a clear and a cloudy day. Where the vegetation is denser the variation is more pronounced.

TABLE I. *Variation in oxygen and carbon dioxide content of waters at East Wareham, Massachusetts*

Source	Date, 1918	Weather	Hour	Temp. C	Carbon Dioxide Cc. per Liter	Oxygen Cc. per Liter
Ditch, State Bog	July 3	Clear	9:00 A.M.	24°	10.1	0.9
“ “ “	“ “	“	11:00 A.M.	25	3.6	2.5
“ “ “			3:00 P.M.	26.5	2.0	4.9
“ “ “	29		9:00 A.M.	25	6.4	2.4
“ “ “			11:00 A.M.	27	5.2	3.6
“ “ “			2:30 P.M.	29	4.5	5.0
“ “ “	“ “	“	4:00 P.M.	29.5	4.0	5.1
“ “ “	Aug. 10	Cloudy	11:00 A.M.	20	5.1	1.8
“ “ “	“ “	“	1:45 P.M.	20.5	3.9	3.6
Spectacle Pond	July 15	Clear	9:00 A.M.	21°	0.7	6.1
“ “	“ “	“	11:00 A.M.	22	0.7	6.1
“ “	“ “	“	3:00 P.M.	24	0.4	6.1
“ “	“ 17	“	10:30 A.M.	24	0.3	6.1
“ “	“ “	“	3:00 P.M.	26	0.2	6.5
“ “	“ 30	Cloudy	9:00 A.M.	25	0.3	5.4
“ “	“ “	“	11:00 A.M.	26	0.2	5.4
‘ “	‘ “					
“ “	“ “	“	2:30 P.M.	26	0.1	5.7
“ “	“ “	“	4:00 P.M.	26	0.1	5.9
Cedar Pond	Aug. 1	Clear	9:30 A.M.	22	0.5	4.5
“ “	“ “	“	“ “ “	22	0.5	4.9
“ “	“ “	“	3:00 P.M.	29	0.4	5.1
“ “	“ “	“	“ “ “	29	0.45	5.5
“ “ (east end)	“ 6	“	9:30 A.M.	22	2.8	3.3
“ “	“ “	“	4:00 P.M.	27	1.1	4.8

The carbon dioxide content of the water in Cedar Pond and in the ditches of the State Bog is much higher than that of the water in Spectacle Pond. This is due to the large amount of decaying organic matter which is constantly giving off carbon dioxide. The carbon dioxide content of the water of Cedar Pond and the State Bog ditches is higher and the oxygen content lower in the morning for three reasons: First, because photosynthesis does not take place during the night and carbon dioxide is not withdrawn by the plants. Second, respiration is going on, as a result of which carbon dioxide is given off and oxygen taken up. Third, the organic matter on the bottom is slowly taking up oxygen and liberating carbon dioxide. This applies also in cloudy weather.

Even after these facts had been ascertained, their significance as a factor in cranberry culture was not fully appreciated until the spring of 1919. Attention was then called forcibly to the importance of weather conditions in relation to the oxygen content of flooding water through the difficulty which many of the growers experienced in injury to the buds and new shoots of cranberry plants, resulting from the flooding of bogs during a period of cloudy weather. Although data had already been obtained which would apparently account for injury under such circumstances, it seemed advisable to make further investigations upon this point.

Dr. H. J. Franklin, of the Massachusetts State Cranberry Station at East Wareham, had planned some experiments with cranberries to determine whether or not reduction of light could cause injury to cranberry blossoms or tips. Four pieces of cranberry sod were dug up and placed in galvanized iron tubs. Two of these tubs were filled with ditch water and set in the main ditch of the State Bog so that the edge of the tub was an inch or more above the surface of the water in the ditch. This prevented the entrance of any water from the outside. One of the tubs was covered with pieces of corrugated metal roofing to exclude light, while the other was left exposed. Two other tubs containing pieces of cranberry sod were similarly placed in Spectacle Pond.

The experiment began in the late afternoon of June 28, 1919. Analyses for the oxygen content of the water in each of the four tubs, in the main ditch, and in Spectacle Pond were made at the beginning of the experiment. Other analyses were made at sun-down, at 10:00 to 11:00 p.m., before sunrise the next morning, and two or three times during the day. This general procedure was followed throughout the experiment although samples were not taken as frequently after the second day. The experiment extended through four days for the vines in Spectacle Pond. The vines in the ditch were taken out after three days. The results of the analyses for oxygen are presented in figures 1, 2, and 3. The determinations of oxygen content were made by the Winkler (7, p. 2843) method.

The results were strikingly clear. The vines in the shaded tub in the ditch had all, or nearly all, of the blossoms and many of the growing tips either killed or injured. On the vines in the unshaded tub in the ditch almost no injury was evident. In the set of tubs in Spectacle Pond the same thing occurred. The plants in the shaded tub were obviously seriously injured while the vines in the unshaded tub escaped with little or no apparent injury. It will be observed on consulting the graphs that the oxygen content of the water in the shaded tubs both in Spectacle Pond and in the ditch dropped very rapidly after the experiment was begun and remained at a very low and essentially constant level throughout the course of the experiment. That the oxygen content did not reach a point of absolute deficiency at any time is probably to be accounted for by the fact that oxygen is taken up slowly from the air.

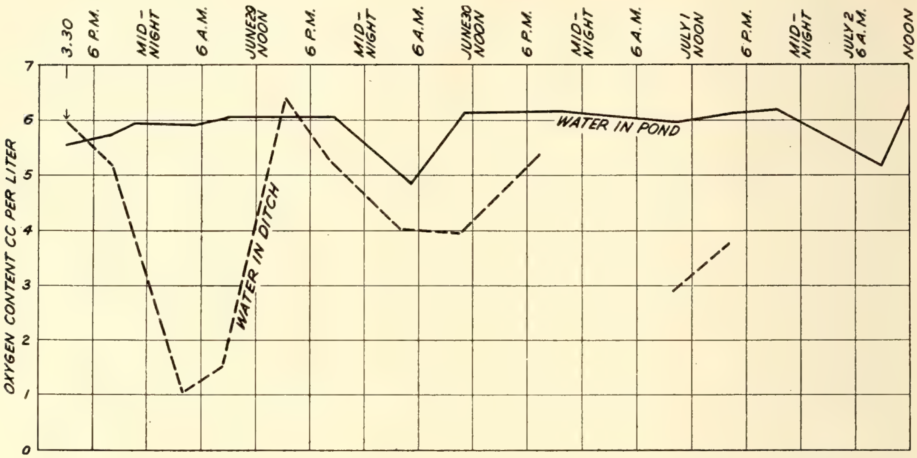


FIG. 1. Oxygen content of water from Spectacle Pond and the State Bog ditch, East Wareham, Massachusetts, 1919.

The oxygen content of the unshaded tubs, both in Spectacle Pond and in the ditch, showed considerable variation between the day and night periods (figs. 2 and 3). The variation between day and night was very uniform throughout the experiment, the oxygen content of water in the unshaded tub in Spectacle Pond running slightly higher than in the unshaded tub in the ditch. The oxygen content of the water in Spectacle Pond showed very little variation from day to day or between day and night (figs. 1 and 2) at any time during the experiment. Only on two occasions was a considerable change in the oxygen content of the pond water observed. One of these was at five o'clock on the morning of June 30, the other at nine o'clock on July 2. The decrease in the oxygen content of the water at these times is due to the fact that the water samples were taken too close to the outlet ditch from the State Bog, so that the sample contained a considerable portion of bog water mixed with the pond water. As the bog water contains considerably less oxygen, the result of mixing it with the pond water is to reduce the oxygen content of the latter.

The ditch water also showed a wide variation between day and night in this experiment. This is due to the presence of algae in the water, since these plants during the day were actively giving off oxygen and were using it up during the night in respiration. The lack of vegetation in Spectacle Pond accounts for the uniformity of the oxygen content as between day and night, the slight changes occurring being due mostly to the difference in temperature.

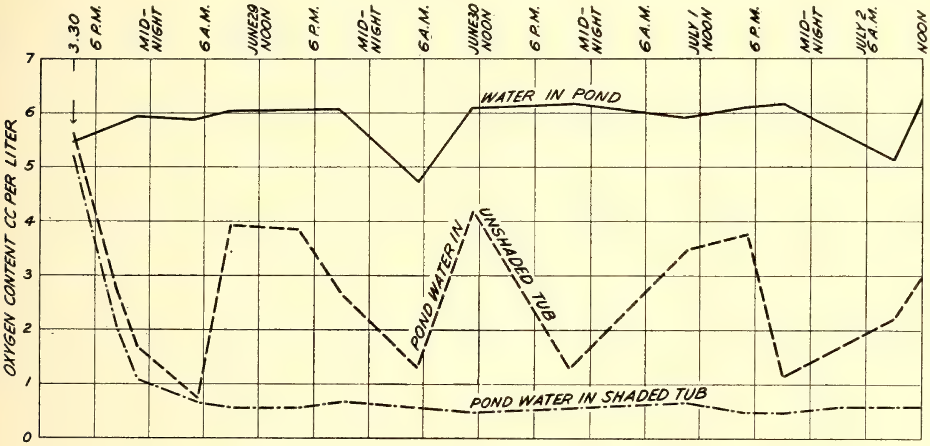


FIG. 2. Oxygen content of water from Spectacle Pond, East Wareham, Massachusetts, and of water from Spectacle Pond held in tubs under experimental conditions, 1919.

From this experiment it is evident that injury occurred only to the plants in the shaded tubs, the water of which shows a great reduction below the oxygen content of either pond or ditch water (figs. 2 and 3). As the oxygen content was the only factor which was changed extensively throughout the experiment, the injury must be attributed to the lack of oxygen.

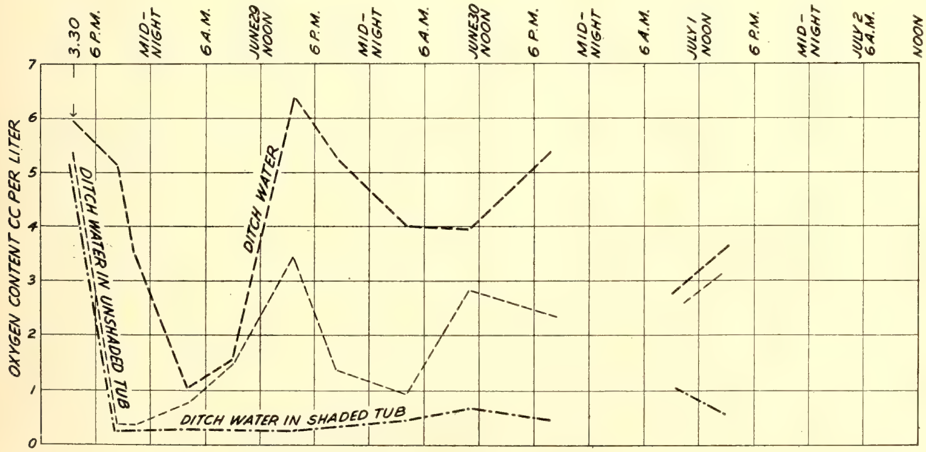


FIG. 3. Oxygen content of water in ditch of State Experiment Bog, East Wareham, Massachusetts, and of water from the ditch held in tubs under experimental conditions, 1919.

Very little difference was noted as to degree of injury between shaded plants in ditch water and shaded plants in pond water. This indicates that nothing in the quality of the water aside from lack of oxygen caused the injury. The plants that were submerged but not shaded suffered little or no injury. Analyses of the water show that in these tubs the oxygen content decreased only during the night and did not remain long at a low level. The shading during the day prevented the accumulation of oxygen which would have carried the plants through a considerable part of the night. Cloudiness would have the same effect, the effect being more pronounced with greater reductions of light.

In this connection, attention may be called to the rate of respiration of the flowers and of the young and old shoots of cranberry plants as measured by the rate of production of carbon dioxide per unit of weight material. The experiments were made with Early Blacks. A weighed amount of flowers, growing tips, and old shoots was placed in closed receptacles of known volume. Determinations of carbon dioxide were made at definite intervals after the experiment was started. Analyses were made with an Allen-Moyer Orsatt apparatus. The readings were corrected to show the volume of dry gas at 0° C. and at a pressure of 760 mm. The results are given in table 2.

Table 2. *A comparison of the carbon dioxide production of flowers, growing tips, and old shoots of cranberries*

Date	Material	Weight in Grams	Volume in Cc.	Duration of Experiment	Temp. C.	Carbon Dioxide		
						% Observed	Cc. per Hour	Cc. per Hour per 100 G,
July 7	Flowers	11.0	25	2 h. 15 m.	22°	0.4	4.2	38.6
“ “	Growing tips	19.0	35	2 h. 20 m.	“	0.7	7.1	37.4
“ “	Old shoots	36.5	54	2 h. 15 m.	“	0.5	5.2	14.3
“ 8	Flowers	11.0	25	15 h. 10 m.	“	2.0	2.5	22.0
“ “	Growing tips	19.0	35	“	“	3.0	3.7	19.3
“ “	Old shoots	36.5	54	“	“	2.6	3.3	6.1
“ 14	Flowers	13.7	26	1 hour	25.5	0.45	10.5	76.5
“ “	Growing tips	25.9	39	“	“	0.5	11.6	46.2
“ “	Old shoots	40.5	57	“	“	0.5	11.5	28.3
“ 14	Flowers	13.7	26	2 hours	27.5	0.5	11.5	42.0
“ “	Growing tips	25.0	39	“	27.5	1.0	22.9	45.8
“ “	Old shoots	40.5	57	“	“	0.6	13.7	16.9
“ 14	Flowers	13.7	26	“	28.0	0.5	11.5	41.8
“ “	Growing tips	25.0	39	“	“	0.9	20.6	41.0
“ “	Old shoots	40.5	57	“	“	0.5	11.5	14.0

From the results of these experiments it is evident that under the same temperature conditions the flowers and growing tips show a much higher rate of production of carbon dioxide than do the old shoots. Nicolas (4, p. 109), in some twenty plants studied, found respiration more rapid and the respiratory quotient higher in young leaves than in those fully matured. Most of the experiments indicate (see table 2) that the flowers produce carbon dioxide at a slightly higher rate than the growing tips, although in the second trial on July 14 this is not true. In these experiments the flowers and growing tips produced carbon dioxide two to three times as fast as did the old shoots. Maige (3, p. 1) has shown in experiments with a large number of plants that the intensity of respiration of floral organs is greater than that of leaves. A more rapid rate of respiration, however, means a greater oxygen requirement. This accounts for the injury to the flowers and growing tips resulting from prolonged submergence in water deficient in oxygen.

From the data submitted it is evident that injury is most apt to occur to a bog by flooding during a period of cloudy weather. Naturally the injury is apt to be greater the longer the period of time during which the water is held on the vines, and especially if cloudiness prevails throughout the period. The degree of injury is probably not directly proportional to the reduction in light intensity, for, as Brown and Heise (2, p. 85) point out, the published works on carbon dioxide assimilation "indicate a progressively smaller augmentation of the rate of assimilation for each increase in light intensity." A certain, as yet unknown, reduction in light intensity is necessary to bring about a balance between the photosynthetic and the respiratory activity. After this point has been reached, accepting the conclusions of Brown and Heise (2, p. 94), each decrement in light intensity should have a progressively greater injurious effect.

A number of other factors which may affect the result are operative. Much depends upon the character of the water as it is placed on the bog. Clear pond or river water has a higher initial oxygen content, so that the oxygen is not depleted as rapidly as from a water supply initially deficient in oxygen. Where water from a cedar swamp, or other reservoir having a great deal of organic matter on the bottom, is used, the initial oxygen content may be very low. This, as indicated elsewhere, is due to the fact that organic matter absorbs oxygen and gives off carbon dioxide. In cloudy weather the initial oxygen content of water from a swamp reservoir would be considerably lower than in clear weather. All other factors remaining the same, greater injury would result during a period of warm weather than of cool, due to the increase in the rate of respiration with a rise of temperature. On the other hand, the ability of the water to absorb oxygen diminishes with an increase of temperature.

In conclusion it may be pointed out that the matter of oxygen content as affected by light intensity and other factors is of great importance in combating insect pests by flooding. A combination of factors producing a low oxygen content in the flooding water at a time when the insect larvae are active is most desirable. Such conditions would, however, be highly detrimental to the cranberries. It would be necessary, therefore, to consider the state of activity of the cranberries and of the insect larvae, and, if possible, to adjust the length of the flooding period so that the larvae would be killed without injury, or at least without extensive injury, to the vines. A careful study of these problems is highly desirable.

Summary

In a study of flooding water of cranberry bogs of Massachusetts and Wisconsin a variation in oxygen and carbon dioxide content of the water was observed.

The effect of cloudiness on the oxygen and carbon dioxide content of water is indirect, resulting from the action of light on submerged vegetation.

The variation in oxygen and carbon dioxide content of flooding water in the cranberry region of Cape Cod, Massachusetts, as affected by light intensity, organic matter, and abundance of vegetation, is shown.

An experiment is described showing the effect of shading submerged cranberry vines. The resulting injury is due to reduction of oxygen content of the water. No essential difference was observed between the amount of injury to shaded vines in pond water and that to shaded vines in bog ditch water.

The flowers and growing tips of shoots, which were the parts most seriously affected, have a higher respiratory rate than old shoots, as shown by experimental results. This accounts for their greater injury by submergence in water deficient in oxygen.

Injury is most apt to occur to a bog by flooding during cloudy weather.

Bureau of Plant Industry,
Washington, D. C.

LITERATURE CITED

1. **Birge, E. A., and Juday, C.** The inland lakes of Wisconsin. The dissolved gases of the water and their biological significance. Wis. Geol. and Nat. Hist. Survey Bull. 22. 1911.
2. **Brown, W. H., and Heise, G. W.** The relation between light intensity and carbon dioxide assimilation. Philip. Jour. Sci. **12**: 85–95. 1917.
3. **Maige, G.** On the respiration of different floral organs. Ann. Sci. Nat. Bot. IX. **14**: 1 (not seen). Abstr. in Exp. Sta. Rec. **25**: 729. 1911.
4. **Nicolas, G.** The variation in the respiration of plants in proportion to age. Bull. Soc. Nat. Hist. Afrique Nord 1910: 109 (not seen). Abstr. in Exp. Sta. Rec. **26**: 628. 1912.
5. **Pettersson, O., and Sonden, K.** Über das Absorptionsvermögen des Wassers für die atmosphärischen Gase. Ber. Deutsch. Chem. Ges. **22**: 1439. 1889.
6. **Roscoe and Lunt.** Chem. Soc. Journ. **55**: 552. 1889.
7. **Winkler, L. W.** Die Bestimmung des im Wasser gelösten Sauerstoffes. Ber. Deutsch. Chem. Ges. **21**: 2843. 1888.

BOTANICAL SOCIETY OF AMERICA

Reorganized 1906, uniting the Botanical Society of America (1893), the Society for Plant Morphology and Physiology (1896), and the American Mycological Society (1903).

OFFICERS FOR 1921

President: C. E. Allen
University of Wisconsin

Vice-President: H. C. Cowles
University of Chicago

Secretary: J. R. Schramm
Cornell University, Ithaca, N. Y.

Treasurer: Irving W. Bailey
Bussey Institution, Harvard University

Councilors:

N. L. Britton
New York Botanical Garden

F. C. Newcombe
University of Michigan

William Trelease
University of Illinois

J. C. Arthur
Purdue University

Any one actively interested in botanical work is eligible for membership. Candidates for membership are recommended by three members, not members of the Council, on blanks to be obtained from the Secretary.

The annual dues are $5.00, including subscription to the American Journal of Botany. Candidates proposed for membership, and approved by the Council, may receive one copy of the Journal at $4.00 per year pending their election at the winter meeting of the Society.

A Fair And Frank Way To Buy Your Greenhouses

You and I know full well that salesmen have a way of putting the best foot forward, so to speak. In which particular, ours are quite like the rest. They honestly believe we build the best that's built.

Their belief, however, is not based so much on what we have told them as on what they have seen of our houses and our way of doing things.

What they really sell is their confidence in Hitchings & Company.

After which the buyer himself sells the greenhouse to himself.

The shortest short cut to that point of satisfaction, is to go and see some Hitchings houses and talk with their owners.

With that in mind let us send you names of some near-by houses.

We will gladly go with you, however, that is as you may incline.

Hitchings and Company

General Office and Factory: ELIZABETH, N. J.

NEW YORK
1170 Broadway

BOSTON-9
294 Washington St.

Vol. VIII FEBRUARY, 1921 No. 2

AMERICAN JOURNAL OF BOTANY

OFFICIAL PUBLICATION OF THE
BOTANICAL SOCIETY OF AMERICA

Smithsonian Institution
MAR 29 1921
National Museum

CONTENTS

Influence of temperature on the relations between nutrient salt proportions and the early growth of wheat . . . . W. F. GERICKE 59

The vascular anatomy of dimerous and trimerous seedlings of Phaseolus vulgaris.
J. ARTHUR HARRIS, EDMUND W. SINNOTT, JOHN Y. PENNYPACKER, AND G. B. DURHAM 63

Aspergillus flavus, A. oryzae, and associated species.
CHARLES THOM AND MARGARET B. CHURCH 103

PUBLISHED
IN COOPERATION WITH THE BOTANICAL SOCIETY OF AMERICA
AT 41 NORTH QUEEN STREET, LANCASTER, PA.
BY THE
BROOKLYN BOTANIC GARDEN
BROOKLYN, N. Y.

Entered as second-class matter February 21, 1914, at the post office at Lancaster, Pennsylvania, under the act of March 3, 1879.

AMERICAN JOURNAL OF BOTANY

Devoted to All Branches of Botanical Science

Established 1914

EDITED BY A COMMITTEE OF THE BOTANICAL SOCIETY OF AMERICA

EDITORIAL COMMITTEE

C. E. Allen, Editor-in-Chief,
University of Wisconsin

William Crocker,
University of Chicago

L. R. Jones,
University of Wisconsin

C. Stuart Gager, Business Manager
Brooklyn Botanic Garden

Orland E. White,
Brooklyn Botanic Garden

R. A. Harper,
Columbia University

Jacob R. Schramm,
Cornell University

C. L. Shear, *Bureau of Plant Industry*
(Representing American Phytopathological Society)

The Journal is published monthly, except during August and September. Subscription price, $6.00 a year. Single copies 75 cents. Back numbers, 75 cents each; $6.00 a volume, postage extra. Postage will be charged to all foreign countries, except Mexico, Cuba, Porto Rico, Panama Canal Zone, Republic of Panama, Hawaii, Philippine Islands, Guam, Samoan Islands, and Shanghai. Postage to Canada, 20 cents a volume on annual subscriptions; to all other countries in the Postal Union, 40 cents a volume on annual subscriptions.

The pages of the Journal are open to members of the Botanical Society of America, or to candidates approved for membership.

Manuscript offered for publication should be typewritten, and should in all cases be submitted to the Editor-in-Chief.

Papers are limited to 20 pages in length, exclusive of plates, except that additional pages may be arranged for at cost rate to authors (approximately $3.00 a page).

Proofs should be corrected immediately on receipt, and returned to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y.

Illustrations, other than zinc etchings, and tabular matter should, in general, be restricted to approximately ten percent of the number of pages of text. Authors may be charged at cost rates for illustrations and tabular matter in excess of that amount (approximately $6.40 a page for tabular matter; 15 cents per square inch for half-tones).

Separates should be ordered when proof is returned. Ffty copies without cover will be supplied free; cover and additional copies at cost.

Remittances should be made payable to American Journal of Botany. Ten cents must be added to all checks not drawn on New York City banks.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

Correspondence concerning editorial matters should be addressed to Prof. C. E. Allen, University of Wisconsin, Madison, Wisconsin.

Business correspondence, including notice of change of address, and directions concerning reprints, should be addressed to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y., or 41 North Queen Street, Lancaster, Pa.

AMERICAN
JOURNAL OF BOTANY

VOL. VIII FEBRUARY, 1921 No. 2

INFLUENCE OF TEMPERATURE ON THE RELATIONS BETWEEN NUTRIENT SALT PROPORTIONS AND THE EARLY GROWTH OF WHEAT

W. F. GERICKE

(Received for publication August 5, 1920)

Russell[1] has stated that "Potash-starved plants are the first to suffer in a bad season or to succumb to disease. The Broadbalk wheat plots receiving potassium salts give conspicuously better results than others whenever the year is unfavorable to plant growth. . . . The badness of the season may be connected with high rainfall and correspondingly low temperatures." This statement emphasizes the fact that climatic conditions may exert no inconsiderable influence upon what may be regarded as the best set of proportions of the nutrient salts in the medium in which plants are rooted. What is a good set of salt proportions with one set of climatic conditions may not be a good one with another climatic complex, etc. Results obtained from an experimental study carried out in 1918–19, in the Laboratory of Plant Physiology of the Johns Hopkins University,[2] seem to bear on this general and important proposition, and some of these results are here reported in a preliminary way.

The investigation was planned to bring out the relations between maintained temperature, on the one hand, and the physiological properties of various nutrient solutions, on the other. The germination and early seedling phases in the development of "Marquis" wheat were studied, the grains being supported on paraffined mosquito netting held just beneath the surface of the solution, which was renewed every 24 hours. The containers were glass tumblers having a capacity of 300 cc. Twenty-five seeds were used for each test, and all tests have been repeated at least once. Seven

---

[1] Russell, E. J. Soil conditions and plant growth. 2d ed. 1915 (pp. 42, 43). 3d ed. 1917 (pp. 43, 44).

[2] This study was carried on as part of a cooperation between the committee on salt requirements of representative agricultural plants, of the Division of Biology and Agriculture of the National Research Council, and the laboratory named above. It was partially financed from the war-emergency funds of the Council. The writer wishes to express his appreciation of much kindly interest and advice received from Prof. B. E. Livingston.

[The Journal for January (8: 1–58) was issued March 9, 1921.]

different maintained temperatures were employed, and light was excluded from the experiment chambers. The nutrient solutions used were the 126 3-salt solutions described by the committee on salt requirements,[3] and each solution was tested for every one of the seven different temperatures. These 3-salt solutions are of 6 types;[4] according to the salts employed, and 21 different solutions were tested for each type, each of these having its own peculiar set of salt proportions. The familiar triangular diagram was used to represent the difference in salt proportions. The salts employed were the nine possible combinations of the following six chemical units; K, Ca, Mg, NO_3, H_2PO_4, and SO_4. No iron was added to any of these solutions. As to total concentration, the solutions were all about alike and very weak, being only one tenth as concentrated as the corresponding 1-atmosphere solutions described in the plan above referred to.

The present paper will be confined to certain points brought out for the two temperatures 28° C. and 17° C. (one about optimum and the other distinctly below the optimum temperature for the early growth phases of wheat). Only those solutions of each of the six types tested will be considered that gave the best growth values, determined by the criteria of total shoot elongation per culture and average elongation per seedling of the cultures, for a period of 110 hours, beginning with the placing of the seeds on the net. The best values obtained for the set of 21 solutions for each of the six types for each of the different temperatures tested were considered the "good" solutions, if their growth values obtained lay within the upper one fourth of the total range of values for the same temperature and the same solution type. For example, if the 21 solutions of the type containing the salts KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$, gave a growth value ranging from 1.00 to 1.80 for the average of both the criteria used, tested at a temperature of 28° C., then those solutions whose value lay between 1.60 and 1.80 would be classed as the "good" solutions for the type at that given temperature. The total-shoot-elongation value simply represented the total growth obtained from a solution. The average elongation value per seedling per culture was obtained by dividing the total shoot elongation in centimeters by the number of seedlings the culture contained. The reason these two criteria were employed was to offset any appreciable error that may accrue in the growth value from a failure of some of the seeds to germinate. The following table shows what solutions belong to the "good" class for each type and for each of the two temperatures here dealt with. In the solution designations, which refer to the triangular diagrams, the number following the letter "R" indicates the number of eighths (of the total molecular concentration) that are due to the potassium salt, the number following the letter "S" indicates the number of eighths due to the calcium salt, and the difference between 8 and the sum of these two numbers is the number of eighths due to the magnesium salt.

---

[3] Committee on salt requirements of representative agricultural plants, Division of Biology and Agriculture, National Research Council. A plan for cooperative research on the salt requirements of representative agricultural plants. Edited by Burton E. Livingston. 2d ed. Baltimore, 1919.

[4] For an outline of the chemical scheme of these six types, on which the committee's plan was based, see: Livingston, B. E., and Tottingham, W. E. A new three-salt nutrient-solution for plant cultures. Amer. Jour. Bot. 5: 337–346. 1918.

TABLE 1. *Good nutrient solutions of the six different salt types tested at two different maintained temperatures*

Temperature	Type I KH_2PO_4, $Ca(NO_3)_2$, $MgSO_4$	Type II K_2SO_4, $Ca(NO_3)_2$, $Mg(H_2PO_4)_2$	Type III KNO_3, $Ca(H_2PO_4)_2$, $MgSO_4$	Type IV K_2SO_4, $Ca(H_2PO_4)_2$, $Mg(NO_3)_2$	Type V KNO_3, $CaSO_4$, $Mg(H_2PO_4)_2$	Type VI KH_2PO_4, $CaSO_4$, $Mg(NO_3)_2$
28° C.	R1S3 R1S4 R3S4 R4S1 R4S2	R1S3 R1S4 R2S1	R1S3 R1S4 R1S6 R2S1 R2S2	R1S4 R1S5	R1S3 R1S5 R2S1	R1S3 R1S4 R3S4 R4S2 R4S3 R5S1 R5S2
17° C.	R3S2 R3S3 R4S1 R4S3 R5S1	R3S3 R5S1 R5S2	R2S5 R3S4 R4S1 R5S1	R4S1 R5S1 R5S2	R5S1 R5S2	R4S3 R5S1 R5S2

It is seen at once that the "good" group of each type of solution comprises from two to seven different solutions. It appears that there is generally a marked difference between the sets of salt proportions that proved good with the higher temperature, on the one hand, and those that proved good with the lower ones, on the other. For types II, III, IV, and V, low partial concentrations (1 or 2 eighths) of the potassium salt characterize the group for 28°, while high partial concentrations (2–5 eighths) of this salt characterize the group for 17°. There is a suggestion of this same generalization for types I and VI also. For type I the potassium salt has partial concentrations of from 1 to 4 units for the higher temperature, while the corresponding values for the lower temperature lie between 3 and 5. Similarly, for type VI, the potassium-salt values lie between 1 and 5 for the higher temperature and between 4 and 5 for the lower. It is thus indicated that the proportion of the potassium salt should be high for the low temperature, and low for the high temperature, if the solution is to give good growth values. Since three different potassium salts are involved, it appears that this suggested generalization really applies to the partial concentration of potassium itself rather than to that of the salt that supplies this element.

It is to be noted that the two types (I and VI) for which this statement concerning potassium is least definitely applicable, are both characterized by the fact that the potassium salt is the phosphate. But, for three other types (II, IV, and V), high partial concentrations of H_2PO_4 characterize the good solutions for the higher temperature, while lower concentrations of H_2PO_4 mark the good ones for the lower temperature. It is thus suggested that the H_2PO_4-relation (to growth and to temperature) may be the reverse of the K-relation. Potassium phosphate being employed in types I and VI as the only source of K as well as of H_2PO_4, a sort of antagonistic effect might be expected in these cases, and this expectation seems to have been realized. A study of the two groups of good solutions for each of these two types suggests an inversion of the H_2PO_4-relation (*low* partial concentrations for the *higher* temperature, etc.) and a masking of the K-relation, as has been mentioned. Type III furnishes no evidence in this regard.

It appears from these results that temperature is of prime moment in determining the mineral requirements for good germination and initial growth in this wheat, at least within the general limits of these experimental tests, and it seems safe to suppose that other climatic conditions may not be without influence. It is suggested that some of the unexplained discrepancies that are commonly encountered in comparative studies on plant salt requirements and on the application of fertilizers to agricultural soils, may be related to climatic influences. It seems clear that all influential conditions should be quantitatively considered in such studies.

THE VASCULAR ANATOMY OF DIMEROUS AND TRIMEROUS SEEDLINGS OF PHASEOLUS VULGARIS

J. Arthur Harris, Edmund W. Sinnott, John Y. Pennypacker, and G. B. Durham

(Received for publication August 21, 1920)

Introductory

The great majority of investigations dealing with the anatomy of plants have been purely descriptive in character. As a result of observation, the typical or average condition of plant structures has been recorded in terms which are general and often indefinite. Comparatively few morphological papers deal with the problem of the variation of the structures under consideration, treat of their correlations with one another, or even present the detailed measurements which might serve for the solution of such fundamental morphological problems.

The older comparative morphology is indispensable. It provides a general knowledge of plant structures and serves as a basis for the classification of the vegetable kingdom. The recognition that description must be supplemented by the results of experimentation has, however, led to the establishment of the newer special science of experimental morphology. The time has come to extend still further our study of plant form by calling to the service of vegetable morphology the methods of measurement and mathematical analysis. These methods are particularly useful in an attack upon the fundamental problems of morphogenesis. It is by measuring exactly the various plant structures during their successive stages of development, in terms of size or number; by determining their relative variability in different organs or regions of the plant, or under varying external conditions, and by discovering such correlations as exist, both among the structures themselves and between them and their progenitors and their environment, that we shall be able to build up a body of fact on which morphogenetic theory may rest.

The present paper gives a portion of the results of a biometric analysis of a comparatively simple morphological problem, that of the gross vascular anatomy of certain normal and abnormal bean seedlings. Our purpose has been:

1. A study of the vascular anatomy of normal and of abnormal seedlings from the point of view of descriptive morphology—a preliminary which we believe to be essential to a sound interpretation of any statistical results.

2. A statistical study of the number and variation of the vascular elements in different regions of the seedling.

3. An investigation of the correlations between these internal characters (such as those which exist between bundle number in different regions of the seedling) and between the internal characters and external features of the plant.

The results of the first and second phases of the investigation are set forth in the present paper; the third is reserved for a later publication.

Materials and Methods

A priori considerations seemed to indicate that a promising line of attack upon the general field of quantitative plant morphology lay in the investigation of vascular bundle number. Such an investigation should be on a scale sufficiently large to make possible the determination of trustworthy biometric constants, and should have as its subject a plant organ of relatively simple but variable structure. Because of the ease with which they can be grown in quantity, their sharply marked external characteristics, their convenient size for histological work, and their relatively simple internal structure, seedlings of *Phaseolus vulgaris* furnish highly satisfactory material for a study of variation and correlation in vascular structures.

Among the many types of variant seedlings of the garden beans which may be secured by extensive plantings, two were selected for investigation: (*a*) normal (*dimerous*) seedlings, with two cotyledons and two primordial leaves, and (*b*) *trimerous* seedlings, with three cotyledons and three primordial leaves. For brevity in table headings the dimerous plants will sometimes be represented by "2–2" and the trimerous by "3–3," where the first figure gives the number of cotyledons and the second the number of primordial leaves.

Since one of the purposes of this work is to carry out a comparison of bundle number in normal and teratological seedlings, the selection of a satisfactory control series of normal plants is a matter of primary importance. It is essential that the seedlings of the types to be compared be selected in a manner to reduce to a minimum any external influences tending to bring about differences between them. It is clear that if the abnormal and the normal seedlings were taken from different series of parent plants, either genetic differences or environmental influences acting upon the parent plant might be effective in bringing about a differentiation in the characters of the seedling examined. A normal seedling from the same parent was, therefore, taken for comparison with each abnormal seedling[1] in each series in which the seed was derived from individual parent plants. Closer control of the influence of innate differences in the parents and of the possible influence of parental environment hardly seems practicable since the pairs of abnormal and normal seedlings were, in three of the lines investigated, derived from the same parent plant.

---

[1] In the vast majority of the cases one abnormal seedling only was sectioned from a parent plant. When more than one abnormal seedling was available a control was taken for each. Naturally it is immaterial whether control *a* or *b* be compared with abnormal seedling *A* or *B*, since all are siblings.

Furthermore, care was taken that seedlings compared were grown under essentially identical conditions, in order to reduce to a minimum the environmental influences which might possibly tend to bring about differences between them. Seeds from individual plants were germinated in flats and harvested as soon as possible after they broke through the sand. Thus all seeds not only developed under the same parental environment but were germinated under sensibly identical conditions, were collected simultaneously, and were in consequence sectioned at essentially the same stage of maturity.

Because of the rapidity with which seedlings change and the great influence of temperature upon growth, it is difficult to standardize, or exactly to describe, the stage of development at which the seedlings were taken. Most of them were placed in alcohol before or very soon after the primordial leaves had unfolded. Thus a fairly uniform and early stage of development was secured.[2]

Free-hand sections were cut and mounted temporarily. When necessary, phloroglucin and hydrochloric acid were used to bring out the vascular bundles. The general vascular topography of the seedlings was studied, but the data for the statistical analysis of the seedling anatomy were derived from a careful count of the number of vascular bundles at various levels in the seedling. Because of a certain amount of variation in the number of bundles with position in the organ, counts were made in definite regions only—the basal region of the hypocotyl (just at the point of transition from "root structure" to "stem structure"); the median region of the hypocotyl; and the median region of the epicotyl. In three series counts were also made of the protoxylem poles in the upper portion of the primary root.

The number of data available for the several regions differs because of a change in the plan of the work. Sectioning and counting were begun by two of us at Cold Spring Harbor in the summer of 1917 and continued with the assistance of Miss Eunice Kinnear in the summer of 1918. This work was confined to the mid-regions of the hypocotyl and epicotyl. From a statistical study of these data it seemed desirable to have a further series of countings made independently by a specialist in vascular anatomy. The work was, therefore, continued at Storrs during 1918, 1919, and 1920. We are greatly indebted to Miss Flora Miller for assistance in this phase of the work. At Storrs, sections were made at the base of the hypocotyl as well as in the mid-region of hypocotyl and epicotyl. In three series, sections were made of the root as well.

The bundles vary considerably in size, the largest being well developed and the smallest containing only one or two lignified xylem cells and a small patch of phloem. Some are even more reduced, consisting of a phloem patch alone. Any strand in which at least one well lignified xylem element could be made out was counted as a bundle. Some of the bundles are partially double in character, this condition being due either to partial fusion or to incipient division. Whenever such a strand was surrounded by one bundle sheath it was counted as one bundle; when the separation was so great that the bundle sheath itself showed signs of division, the strand was counted as two.

---

[2] Some of the seedlings of line 143 were allowed to become a little older, but there is no evidence of change in bundle number with age.

The seedlings were harvested at a stage when the vascular tissues of the first epicotyledonary internode were not completely lignified, and the number of bundles counted was therefore possibly less than the number which would finally be developed there.

None of these possible sources of error is believed to be great enough to affect the conclusions appreciably.

The Structure of the Seedling

In order to provide a sound basis for the understanding and interpretation of our later work, it is necessary to present a brief descriptive account of the structure of the seedlings.

The Normal (Dimerous) Seedling

The morphology of the seedling of Phaseolus has received the attention of several investigators, notably Dodel[3] and Compton.[4] Like most of the large seedlings of the Leguminosae it is normally tetrarch in fundamental plan; that is, there are four groups of protoxylem elements in the root. At a very early stage there is associated with each of these a group of metaxylem cells. It is these groups of metaxylem elements, throughout the whole seedling, which in the present paper are counted as "bundles," even though (as is sometimes the case) they are not associated with protoxylem clusters.

At the stage when these seedlings were harvested, cambial activity had hardly begun to show itself, so that these primary bundles remained distinct and easy to identify.

The condition in the upper part of the root of a normal seedling is shown in figure 1. The four bundles, two in the cotyledonary plane and two in the intercotyledonary plane, are more or less V-shaped (with the protoxylem group in an exarch position at the apex of the V) and tend to extend laterally. They surround a large pith. In passing up into the base of the hypocotyl, each of these bundles divides into two (fig. 2), and typical stem structure, with the protoxylem in an endarch position, begins to be assumed. Each pair is subsequently referred to as a "primary double bundle." Thus the level of transition from root structure to stem structure is low, being practically at the base of the hypocotyl. The members of each of these four pairs soon separate until the eight bundles are approximately equidistant (fig. 3), a condition which persists throughout the hypocotyl until the cotyledonary node is approached.

---

[3] Dodel. A. Der Übergang des Dicotyledonen-stengels in die Pfahl-wurzel. Pringsh. Jahrb. 8: 149–193. 1872.

[4] Compton, R. H. An investigation of the seedling structure in the Leguminosae. Jour. Linn. Soc. 41: 7–122. 1912.

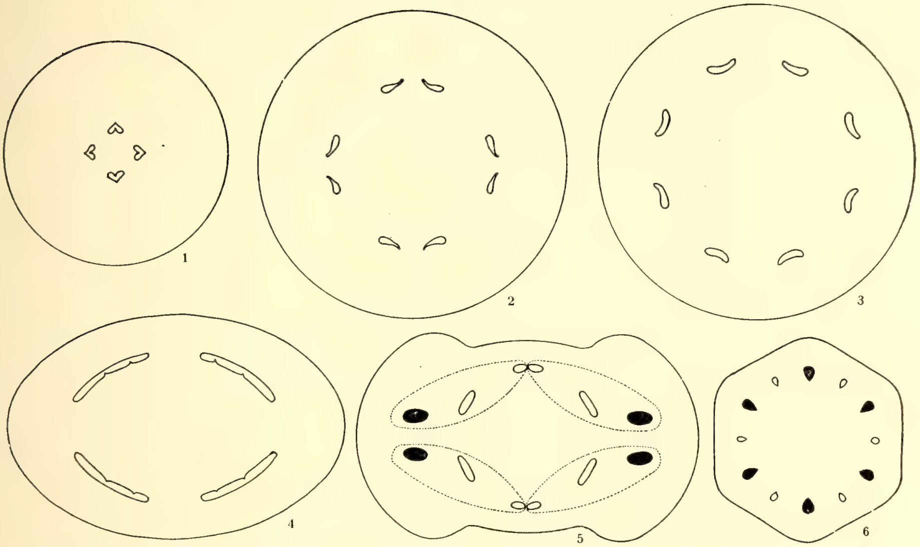


Fig. 1. Dimerous seedling. Transverse section through the root, showing its tetrarch condition (four protoxylem poles). Fig. 2. Dimerous seedling. Transverse section through the base of the hypocotyl showing the four primary double bundles, each of which has been derived from one of the four root strands. Fig. 3. Dimerous seedling. Transverse section through the mid-region of the hypocotyl showing the normal eight-bundled condition. No intercalary bundles are figured. Fig. 4. Dimerous seedling. Transverse section just below the cotyledonary node. The four bundles or bundle groups have originated by a more or less complete fusion of the adjacent members of each of the original pairs. Each bundle, as shown by the two constrictions in it, is about to break up into the three strands shown in figure 5. Fig. 5. Dimerous seedling. Transverse section through the cotyledonary node. Each group of three strands which have arisen by a breaking up of the large bundles in figure 4 is here enclosed by a dotted line. These three strands are a cotyledonary trace (solid black), an epicotyledonary bundle, and a small bundle which will fuse with its adjacent neighbor to form another epicotyledonary bundle. Fig. 6. Dimerous seedling. Transverse section through the mid-region of the epicotyl showing the twelve bundles which have arisen by the splitting of the six original epicotyledonary bundles. The six strands which are to go off as traces to the two primordial leaves are solid black.

In addition to these bundles, there are in a considerable percentage of the normal seedlings studied a variable number of accessory or intercalary bundles, the "Zwischenstränge" of Dodel. These may make their appearance in the upper part of the root or in the lower region of the hypocotyl, some ending blindly below and others arising by division of the primary bundles. These intercalary bundles, which are not a very common feature of seedling anatomy in general, perhaps serve to increase the conductive capacity of the hypocotyl and may be associated with the large size of the seedling. They usually lack protoxylem elements.

At the cotyledonary node there is a rather complex anastomosis of the bundle system. The details of this vary somewhat, but its fundamental features are as follows: The two members of each of the two original pairs of bundles in the cotyledonary plane (that is, opposite the two points where the cotyledons will later arise) become widely separated, and each member fuses with the adjacent member of the intercotyledonary pair (fig. 4). Four large bundles or bundle aggregates are thus produced. Each breaks up immediately, usually into three parts. The lateral member of each group of three which is in the *cotyledonary* plane approaches the corresponding bundle of the next group of three, and these two strands become the cotyledonary traces and enter the base of the cotyledon. The lateral member of each group of three which is in the *intercotyledonary* plane approaches the corresponding bundle of the next group and fuses with it. The changes which are made and the resultant condition at this stage are shown in figure 5. Two strands (solid black) are here departing to each cotyledon, and six bundles are left as the basis for the vascular system of the epicotyl. The details of this nodal complex vary somewhat owing to the different levels at which fusion and separation of bundles take place, and to the presence of intercalary bundles. These intercalary bundles, as they approach the cotyledonary node, fuse with the others and are completely lost, exactly six epicotyledonary strands almost invariably emerging from the complex, quite regardless of the number of intercalary bundles which may have entered it from the hypocotyl. This fact we shall find to be of importance when we consider the statistical relationships of bundle number in hypocotyl and epicotyl.

Above the cotyledons, the six remaining bundles approach one another, closing the cotyledonary gaps and forming a ring, the members of which almost immediately divide. The twelve bundles thus produced (fig. 6) persist throughout the first internode of the epicotyl.

At the first node of the epicotyl are inserted the two primordial leaves. Phaseolus, like other Leguminosae which have been investigated, possesses a trilacunar node, the leaf being supplied by three traces, each of which causes a separate gap in the vascular ring.[5] The two primary leaves therefore remove six of the twelve bundles of the epicotyl (solid black in fig. 6). The six new bundles which appear just above the cotyledonary node are, therefore, evidently downwardly extending leaf traces. These facts make

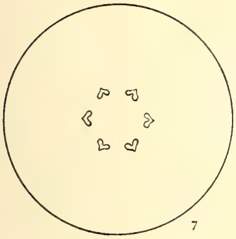
[5] Sinnott, E. W. The anatomy of the node as an aid in the classification of Angiosperms. Amer. Jour. Bot. 1: 303–322. 1914.

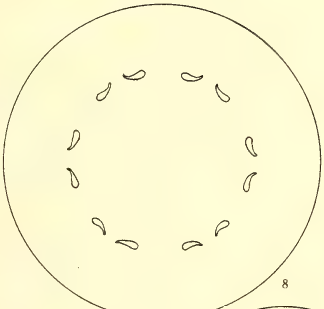
understandable the almost invariably twelve-bundled condition of the first epicotyledonary internode.

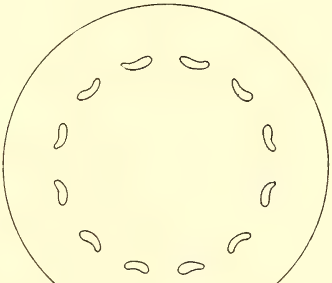
The structure of the normal seedling thus corresponds to the type found by one of the writers[6] to be characteristic of a large number of Angiosperm families, in which the vascular supply to each cotyledon, consisting of two strands, leaves but one gap in the vascular ring; and in which the foliage leaf is trilacunar.

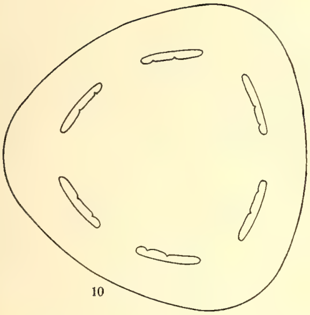
The Trimerous Seedling

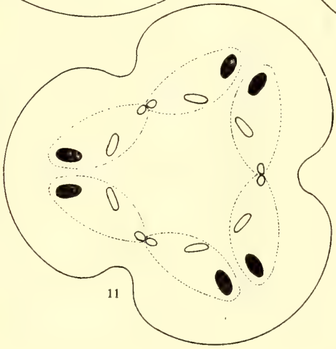
The seedling with three cotyledons and three primordial leaves is built on a different plan from the normal one in that it is prevailingly hexarch, six











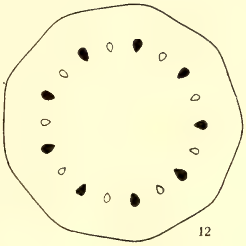


FIG. 7. Trimerous seedling. Transverse section through the root, showing its hexarch condition. FIG. 8. Trimerous seedling. Transverse section through the base of the hypocotyl, showing the six primary double bundles. FIG. 9. Trimerous seedling. Transverse section through the mid-region of the hypocotyl, showing the normal twelve-bundled condition. FIG. 10. Trimerous seedling. Transverse section just below the cotyledonary node. The six bundles or bundle groups correspond in origin and character to the four bundles of the dimerous seedling at this level. FIG. 11. Trimerous seedling. Transverse section through the cotyledonary node. Each group of three strands bounded by a dotted line corresponds in origin and character to a similar group at this level in the dimerous seedling. FIG. 12. Trimerous seedling. Transverse section through the mid-region of the epicotyl, showing the eighteen bundles which have arisen by the splitting of the nine original epicotyledonary bundles. The nine strands which are to go off as traces to the three primordial leaves are solid black.

---

[6] Sinnott, E. W. Conservatism and variability in the seedling of dicotyledons. Amer. Jour. Bot. 5: 120–130. 1918.

bundles occurring in the upper part of the root (fig. 7). This number is soon reduced to five and eventually to four, in passing down the root.

Passing upward into the hypocotyl, the six main strands (the primary double bundles) divide to produce twelve (figs. 8 and 9). Intercalary bundles are much less common than in the normal seedlings, appearing in only a small percentage of cases, and then being rarely more than one or two in number. At the node the same general procedure is followed as in the normal seedling, except, of course, that there are more bundles concerned. Bundles of adjacent pairs approach and fuse (fig. 10). Each of these bundles or bundle aggregates then divides, generally into three. Three cotyledons are each supplied with two bundles (solid black), and three sets of three bundles each—each formed by the fusion of two lateral bundles in the intercotyledonary plane—remain behind. The bundle changes and the final condition at the departure of the cotyledonary traces are shown in figure 11. The epicotyledonary ring which forms from the bundles which remain thus consists of nine strands instead of the normal six. Many of these divide at once, although the number is not usually doubled, as in normal seedlings, but varies from 12 to 18 or even more in the mid-region of the epicotyl (fig. 12). The bundles are much more crowded than in the normal seedlings, which may perhaps account for the failure of some of them to divide at once.

A study of the first epicotyledonary node shows that three strands are given off to each primary leaf, leaving from 6 to 9 in the stem.

It is therefore evident that within classes of seedlings which are uniform externally there are considerable anatomical variations and that the two classes investigated are profoundly differentiated in their anatomical organization.

Our next task is to subject the mass of data upon which these general conclusions are based to a statistical analysis with the object of bringing out otherwise undeterminable relationships.

Bundle Number and its Variation at Different Levels in the Seedlings

From the statistical side we have two problems to consider.

The first is that of the relative numbers of bundles at different levels, *i.e.*, in the root, at the base of the hypocotyl, in the central region of the hypocotyl, and in the epicotyl of the same plant in both normal and abnormal plants, together with the variability in bundle number in different regions.

The second is that of the differences in bundle number, and in variation of bundle number, between normal and abnormal plants.

Since it is impossible to consider type and variation of bundle number at different levels without noting differences in the trimerous and dimerous forms upon which the observations were based, we shall devote this section primarily to a parallel discussion of both problems.

We shall consider in order the levels at which sections were made, beginning at the root.

1. *Root.* Roots were sectioned in the cases of lines 93, 139, and 143. The numbers of bundles[7] in the roots of normal and trimerous seedlings of these lines are shown in table 1.

Table 1

Primary Double Bundles	Line 93		Line 139		Line 143	
	Trimerous	Dimerous	Trimerous	Dimerous	Trimerous	Dimerous
3......	—	—	2	—	4	—
4......	31	132	15	149	37	219
5......	87	20	53	1	113	2
6......	34	—	36	—	66	—
7......	—	—	—	—	1	—

The entries in this table show that most of the normal plants are tetrarch, although a small percentage are pentarch. In the trimerous seedlings the highest percentage are pentarch, but the remainder are distributed between tetrarch and hexarch with a few in more extreme classes. Sections made at progressively lower levels in the root show that the hexarch and pentarch conditions, in the trimerous seedlings, soon give way to tetrarch. This fact doubtless explains the relatively large number of non-hexarch cases

Table 2. *Vascular formula for base of hypocotyl of trimerous seedlings and their normal controls*

Base of Hypocotyl	Line 75		Line 93		Line 98		Line 139		Line 143	
	Trimerous	Dimerous	Trimerous	Dimerous	Trimerous	Dimerous	Trimerous	Dimerous	Trimerous	Dimerous
(4)	—	69	—	34	—	97	—	138	2	150
(4) + 1	—	30	—	37	—	43	1	9	3	55
(4) + 2	—	10	—	13	—	23	—	—	—	4
(4) + 3	—	4	—	5	—	2	—	—	—	—
(4) + 4	—	2	1	1	—	—	—	—	—	—
(4) + 5	—	2	—	—	1	—	—	—	—	—
(4) + 6	1	—	—	—	—	—	—	—	—	—
(5)	1	13	5	22	4	6	4	1	15	5
(5) + 1	8	4	10	18	6	8	4	2	31	5
(5) + 2	2	1	3	9	1	1	—	—	—	—
(5) + 3	—	1	—	1	—	1	—	—	—	—
(6)	107	5	120	10	160	1	92	—	134	—
(6) + 1	12	1	11	3	10	—	5	—	25	1
(6) + 2	2	—	1	2	—	—	—	—	—	—
(7)	7	—	4	—	1	—	—	—	5	1
(7) + 1	—	—	—	—	—	—	—	—	4	—
(7) + 2	1	—	—	—	—	—	—	—	—	—
(8)	1	—	—	—	—	—	—	—	1	—
(8) + 1	—	—	—	—	—	1	—	—	1	—
	142	142	155	155	183	183	106	150	221	221

---

[7] Where the bundles were united in a ring, the number refers to number of protoxylem strands.

observed, for the zone within which the hexarch condition persists is narrow and its level is variable; and there is necessarily more or less variation in the level at which the sections are cut.

2. *Base of Hypocotyl.* In the series of sections of the base of the hypocotyl made at Storrs, the number of double vascular strands (each of which is derived from a primary root bundle and corresponds to a pole of the root) and the number of intercalary strands were recorded separately. There is no difficulty in distinguishing between these two categories of bundles, since the latter are almost invariably without protoxylem elements and are irregularly placed.

The original data for the five lines are condensed in table 2. The number of bundle pairs (the primary double bundles) is given in parenthesis, and the number of intercalary bundles, if such are present, follows the + sign outside the parenthesis.

There are three outstanding features in this table.

First, the wide range of variation in the number and in the combinations of primary double bundles and intercalary bundles in both normal and abnormal plants observed when reasonably large series of seedlings are sectioned. It is clear that an anatomist who deals with only a few seedlings may obtain an altogether inadequate picture of the conditions which actually prevail in the species under investigation.

Second, notwithstanding the wide range of variation there are conspicuous modal classes in both normal and abnormal seedlings. In the normal plants these fall in all cases on four primary double bundles, without intercalary bundles, or with but one intercalary bundle; and in the trimerous plants, on six primary double bundles without intercalary bundles.

Third, the plants which are externally dimerous and trimerous are also clearly differentiated in internal morphology. The internal characters are, however, transgressive. It is impossible in some cases to distinguish from sections of the hypocotyl base alone between plants which superficially fall into the strictly alternative classes of dimery and trimery.

For purposes of more detailed analysis these formulae must be split up into their component elements.

A. Primary Double Bundles. The distribution of the number of primary double bundles in the five lines considered is shown in table 3 for dimerous and trimerous seedlings. These frequencies, reduced to a percentage basis, are represented graphically in figure 13. This shows that in all five lines the modal number of primary double bundles is two higher in the trimerous than in the dimerous plants. In the dimerous plants the modal class is in all cases 4; in the trimerous seedlings the modal class is 6. There is, therefore, a profound reorganization in the vascular anatomy of the seedling upon the assumption of a trimerous external organization.

Limiting our attention to primary double bundles and judging from modal classes only, an increase of fifty percent in the number of vascular elements is

TABLE 3. *Number of primary double bundles at base of hypocotyl in trimerous and dimerous seedlings*

	4	5	6	7	8	Total
Line 75						
Trimerous	1	11	121	8	1	142
Percent	0.70	7.75	85.21	5.63	0.70	
Dimerous	117	19	6	—	—	142
Percent	82.39	13.38	4.23			
Line 93						
Trimerous	1	18	132	4	—	155
Percent	0.65	11.61	85.16	2.58		
Dimerous	90	50	15	—	—	155
Percent	58.06	32.26	9.68			
Line 98						
Trimerous	1	11	170	1	—	183
Percent	0.55	6.01	92.90	0.55		
Dimerous	165	16	1	—	1	183
Percent	90.16	8.74	0.55		0.55	
Line 139						
Trimerous	1	8	97	—	—	106
Percent	0.94	7.55	91.51			
Dimerous	147	3	—	—	—	150
Percent	98.00	2.00				
Line 143						
Trimerous	5	46	159	9	2	221
Percent	2.26	20.81	71.94	4.07	0.90	
Dimerous	209	10	1	1	—	221
Percent	94.57	4.52	0.45	0.45		

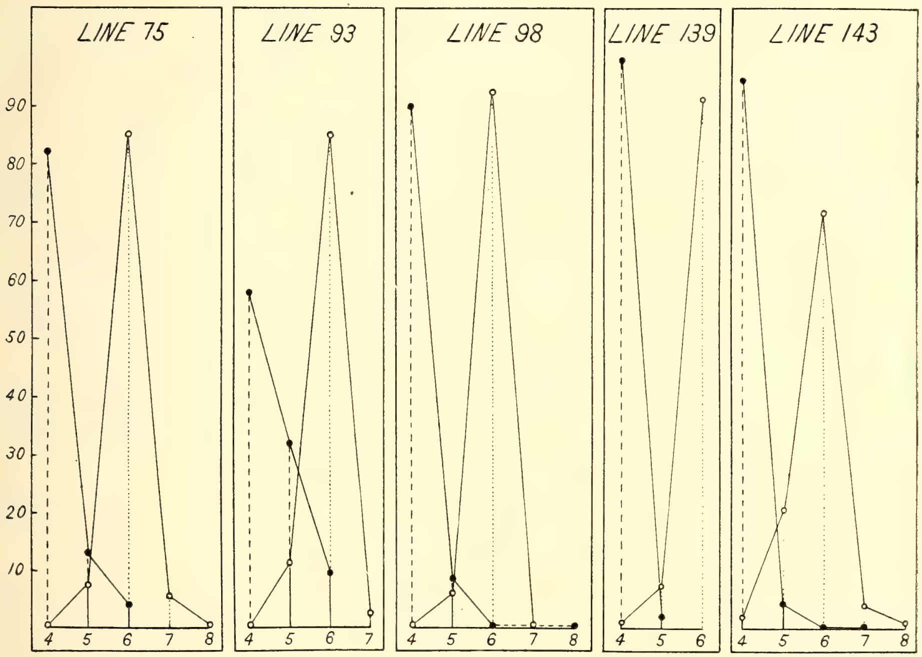


FIG. 13. Percentage frequency distribution for number of primary double bundles at base of hypocotyl in dimerous (solid dots) and trimerous (circles) seedlings.

associated with an increase of fifty percent in the number of cotyledons and leaves. The distributions show, however, that this is only an incomplete, and to some extent an erroneous, statement of the condition. In the dimerous seedlings the modal number of primary double bundles is 4, and all departures from the modal number are higher. In the trimerous seedlings the modal number is 6, and the departures may be in either the positive or the negative direction. The frequency distribution for the dimerous plants is therefore wholly skew, forming a typical J-curve; that for the trimerous plants more or less symmetrical,[8] but with departures occurring chiefly as smaller numbers of bundles.

The variation of primary double bundle number in dimerous and trimerous plants is, therefore, transgressive. The number of externally dimerous seedlings which might be considered to be anatomically trimerous, and the number of trimerous seedlings which might on anatomical grounds be considered dimerous is, however, very small.

Turning to the physical constants in table 4, we note that the mean

TABLE 4. *Statistical constants for number of primary double bundles at base of hypocotyl of trimerous plants and their normal controls*

	Mean	Standard Deviation	Coefficient of Variation
Line 75			
Trimerous (N = 142)	5.98±.02	0.436±.017	7.28±.29
Dimerous (N = 142)	4.22±.03	0.505±.020	11.97±.49
Actual difference	+1.76±.04	−0.069±.026	−4.69±.56
Relative difference	41.71	13.66	
Line 93			
Trimerous (N = 155)	5.90±.02	0.396±.015	6.72±.26
Dimerous (N = 155)	4.52±.04	0.666±.026	14.74±.58
Actual difference	+1.38±.04	−0.270±.030	−8.02±.63
Relative difference	30.53	40.54	
Line 98			
Trimerous (N = 183)	5.93±.01	0.288±.010	4.86±.17
Dimerous (N = 183)	4.12±.02	0.427±.015	10.36±.37
Actual difference	+1.81±.02	−0.139±.018	−5.50±.41
Relative difference	43.93	32.55	
Line 139			
Trimerous (N = 106)	5.91±.02	0.323±.015	5.47±.25
Dimerous (N = 150)	4.02±.01	0.140±.005	3.48±.14
Actual difference	+1.89±.02	+0.183±.016	+1.99±.28
Relative difference	47.01	130.71	
Line 143			
Trimerous (N = 221)	5.81±.03	0.581±.019	10.01±.32
Dimerous (N = 221)	4.07±.01	0.315±.010	7.75±.25
Actual difference	+1.74±.03	+0.266±.021	+2.26±.41
Relative difference	42.75	84.44	

---

[8] Line 139 is probably only an apparent exception to this rule. In both dimerous and trimerous seedlings variations from the modal class are extremely rare, and variations above the modal class have not been found in the 106 trimerous seedlings of this line sectioned.

number of primary double bundles at the base of the hypocotyl of trimerous plants is from 1.38 to 1.89 higher than in the dimerous controls. This represents an excess of from 30.5 to 47.0 percent.

The five lines are not, however, consistent in the relative variability of the normal and abnormal seedlings.

The standard deviation of the number of primary double bundles in the trimerous plants is lower than that in the dimerous plants in lines 75, 93, and 98. The differences are from 13.7 to 40.5 percent of the control values. Lines 139 and 143 are in contrast to the foregoing. The trimerous plants of line 139 have a standard deviation of 0.323 ± .015 bundles, whereas the dimerous controls have a standard deviation of 0.140 ± .005, giving a difference of +.183 ± .016, which is 11.4 times as large as its probable error. In line 143 the trimerous plants have a standard deviation of 0.581 ± .019 bundles as compared with 0.315 ± .010 bundles in the normal controls, giving a difference of +.266 ± .021, which is 12.7 times as large as its probable error. These are relative differences of +130.7 percent for line 139 and + 84.4 percent for line 143.

The same differences in variability between the lines is also conspicuous in the relative variabilities as measured by the coefficients of variation. In the first three lines (75, 93, and 98) the coefficients of variation in the trimerous plants range from 4.9 to 7.3 percent as compared with 10.4 to 14.7 percent in the dimerous controls, giving differences in relative

TABLE 5. *Number of intercalary bundles at base of hypocotyl in trimerous and dimerous seedlings*

	0	1	2	3	4	5	6	Total
Line 75								
Trimerous	116	20	5	—	—	—	1	142
Percent	81.69	14.08	3.52				0.70	
Dimerous	87	35	11	5	2	2	—	142
Percent	61.27	24.65	7.75	3.52	1.41	1.41		
Line 93								
Trimerous	129	21	4	—	1	—	—	155
Percent	83.23	13.55	2.58		0.65			
Dimerous	66	58	24	6	1	—	—	155
Percent	42.58	37.42	15.48	3.87	0.65			
Line 98								
Trimerous	165	16	1	—	—	1	—	183
Percent	90.16	8.74	0.55			0.55		
Dimerous	104	52	24	3	—	—	—	183
Percent	56.83	28.42	13.11	1.64				
Line 139								
Trimerous	96	10	—	—	—	—	—	106
Percent	90.57	9.43						
Dimerous	139	11	—	—	—	—	—	150
Percent	92.67	7.33						
Line 143								
Trimerous	157	64	—	—	—	—	—	221
Percent	71.04	28.95						
Dimerous	156	61	4	—	—	—	—	221
Percent	70.58	27.60	1.80					

variability ranging from –4.7 to –8.0 percent. In line 139 the coefficient of variation for trimerous seedlings is 5.47, whereas that for dimerous seedlings is 3.48. In line 143, the coefficient of variation for trimerous seedlings is 10.01, whereas that for dimerous seedlings is 7.75. Thus the relative variability in these two lines is greater in the *trimerous* than in the *dimerous* seedlings.

B. Intercalary Bundles. The distribution of the number of intercalary bundles (considered alone) in the base of the hypocotyl is shown in table 5.

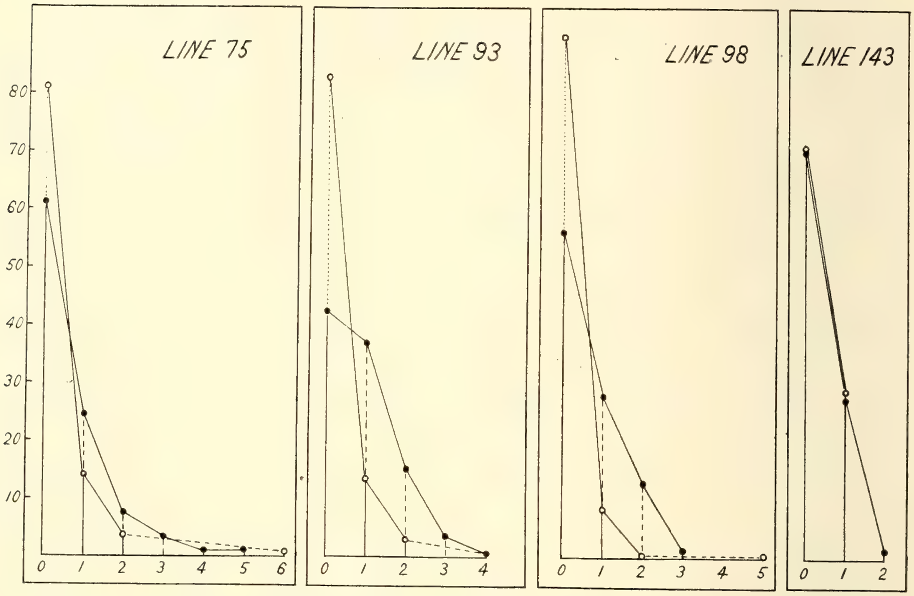


FIG. 14. Percentage frequency distribution of number of intercalary bundles at base of hypocotyl in dimerous (solid dots) and trimerous (circles) seedlings.

The graphs in figure 14 show that for both dimerous and trimerous seedlings *no* intercalary bundles is the modal condition. In both cases the distribution is wholly skew. The normal and the abnormal seedlings of lines 75, 93, and 98 differ conspicuously, however, in that the percentage of seedlings with no intercalary bundles is much higher in the trimerous seedlings, while, conversely, the percentage of seedlings with from 1 to 5 intercalary bundles is much higher in the dimerous plants. These differences are not found in lines 139 and 143. As a matter of fact, the percentage of seedlings with no intercalary bundles is slightly, but perhaps not significantly, higher in the dimerous seedlings of line 139. In both lines 139 and 143 the number of seedlings with 1 or 2 intercalary bundles is very small indeed in both trimerous and dimerous series. The two lines are essentially alike in this regard and line 143 only is represented on the diagram.

The percentages of the seedlings with no intercalary bundles in the two classes of plants and the differences in the percentage are as follows:

	Trimerous	Dimerous	Difference
Line 75	81.69	61.27	+20.42
Line 93	83.23	42.58	+40.65
Line 98	90.16	56.83	+33.33
Line 139	90.57	92.67	− 2.10
Line 143	71.04	70.58	+ 0.46

The physical constants in table 6 show that the mean number of intercalary bundles in both normal and abnormal seedlings is small—less than a single bundle per plant in every case.

TABLE 6. *Statistical constants for number of intercalary bundles at base of hypocotyl of trimerous plants and their normal controls*

	Mean	Standard Deviation	Coefficient of Variation
Line 75			
Trimerous (N = 142)	.25±.04	0.686±.027	270.69±42.86
Dimerous (N = 142)	.63±.06	1.024±.041	161.60±16.13
Actual difference	−.38±.07	−0.338±.049	+109.09±45.79
Relative difference	60.32	33.00	
Line 93			
Trimerous (N = 155)	.21±.03	.545±.021	255.80±36.78
Dimerous (N = 155)	.83±.05	.874±.033	105.79± 7.29
Actual difference	−.62±.06	− .329±.039	+150.01±37.50
Relative difference	74.69	37.64	
Line 98			
Trimerous (N = 183)	.13±.02	.480±.017	381.67±73.88
Dimerous (N = 183)	.60±.04	.776±.027	130.21± 9.62
Actual difference	−.47±.04	− .296±.032	+251.46±74.50
Relative difference	78.33	38.14	
Line 139			
Trimerous (N = 106)	.09±.02	.292±.014	309.84±64.50
Dimerous (N = 150)	.07±.01	.261±.010	355.48±70.95
Actual difference	+.02±.02	+ .031±.017	− 45.64±95.89
Relative difference	28.57	11.88	
Line 143			
Trimerous (N = 221)	.29±.02	.454±.015	156.62±12.21
Dimerous (N = 221)	.31±.02	.501±.016	160.44±12.76
Actual difference	−.02±.03	− .047±.021	− 3.82±17.66
Relative difference	6.45	9.38	

Again the lines fall into two classes, those in which the number of intercalary bundles is conspicuously higher in the dimerous plants (lines 75, 93, and 98) and those in which the numbers are essentially identical (lines 139 and 143). In the trimerous seedlings of the first group the average number ranges from 0.13 to 0.25, whereas in the dimerous it varies from 0.60 to 0.83 bundle. Thus the mean number of intercalary bundles is from 60 to 78 percent smaller in the trimerous than in the dimerous seedlings.

In line 139 the mean number of intercalary bundles is actually larger in the trimerous seedlings, but the difference is only +.02 ± .02.

In line 143 the mean number of intercalary bundles in trimerous and dimerous seedlings is practically identical, the difference being only −.02 ± .03. In both of these lines the differences are insignificant in comparison with their probable errors.

It is also interesting to note that in lines 75, 93, and 98 the differentiation between abnormal and normal seedlings is greater with respect to the number of intercalary bundles than with respect to primary double bundles. Turning back to table 4, we note that the number of primary double bundles is from 31 to 44 percent higher in the trimerous plants, whereas the number of intercalary bundles is from 60 to 78 percent lower. In lines 139 and 143 the difference in the mean of the number of primary double bundles of trimerous and dimerous plants is practically the same as in the other lines, but in these lines the two types of seedlings are essentially identical in number of intercalary bundles.

If we consider the comparative variability of dimerous and trimerous seedlings as to intercalary bundle number, we find that here, as in the case of number of primary double bundles, the lines differ among themselves. In all lines except 139 the standard deviations of number of intercalary bundles in the trimerous seedlings are smaller than in the dimerous. In lines 75, 93, and 98 the constants for the trimerous seedlings are from 33 to 38 percent smaller than those of the dimerous controls. In line 143 the difference has the same sign but is only −9.38 percent of the control value. In line 139 the difference is + 11.88 percent.

The coefficients of variation are very high in both normal and abnormal seedlings, and this great variation renders the probable errors of little value as criteria of statistical significance of differences between the two types of seedlings. In lines 75, 93, and 98, the coefficients of variation for trimerous plants are conspicuously higher than those for the dimerous controls. In line 143 the coefficients of variation for the two types of seedlings are practically the same. In line 139, however, the coefficient of variation for the number of intercalary bundles is higher in dimerous than in trimerous plants.

C. Total Bundles. Having considered the frequency distribution and statistical constants for the two types of vascular structures found in the base of the hypocotyl, it is now desirable to combine the two types of bundles in order to consider the total number of vascular elements at this level.

This problem presents certain morphological difficulties. The primary double bundles are each derived from a single root pole, and do not become clearly divided into two bundles until the level of transition is reached from root structure to stem structure at the base of the hypocotyl. Many of the intercalary bundles appear at this level or a little lower. In determining

TABLE 7. *Total number of bundles at base of hypocotyl in trimerous and dimerous seedlings. Primary double bundles are counted as one bundle only*

	4	5	6	7	8	9	10	Total
Line 75								
Trimerous	—	1	115	21	3	1	1	142
Percent		0.70	80.99	14.79	2.11	0.70	0.70	
Dimerous	69	43	19	6	3	2	—	142
Percent	48.59	30.28	13.38	4.23	2.11	1.41		
Line 93								
Trimerous	—	5	130	18	2	—	—	155
Percent		3.23	83.87	11.61	1.29			
Dimerous	34	59	41	17	4	—	—	155
Percent	21.94	38.06	26.45	10.97	2.58			
Line 98								
Trimerous	—	4	166	12	—	1	—	183
Percent		2.19	90.71	6.56		0.55		
Dimerous	97	49	32	3	1	1	—	183
Percent	53.01	26.78	17.49	1.64	0.55	0.55		
Line 139								
Trimerous	—	5	96	5	—	—	—	106
Percent		4.72	90.57	4.72				
Dimerous	138	10	2	—	—	—	—	150
Percent	92.00	6.67	1.33					
Line 143								
Trimerous	2	18	165	30	5	1	—	221
Percent	0.90	8.14	74.66	13.57	2.26	0.45		
Dimerous	150	60	9	2	—	—	—	221
Percent	67.87	27.15	40	0.91				

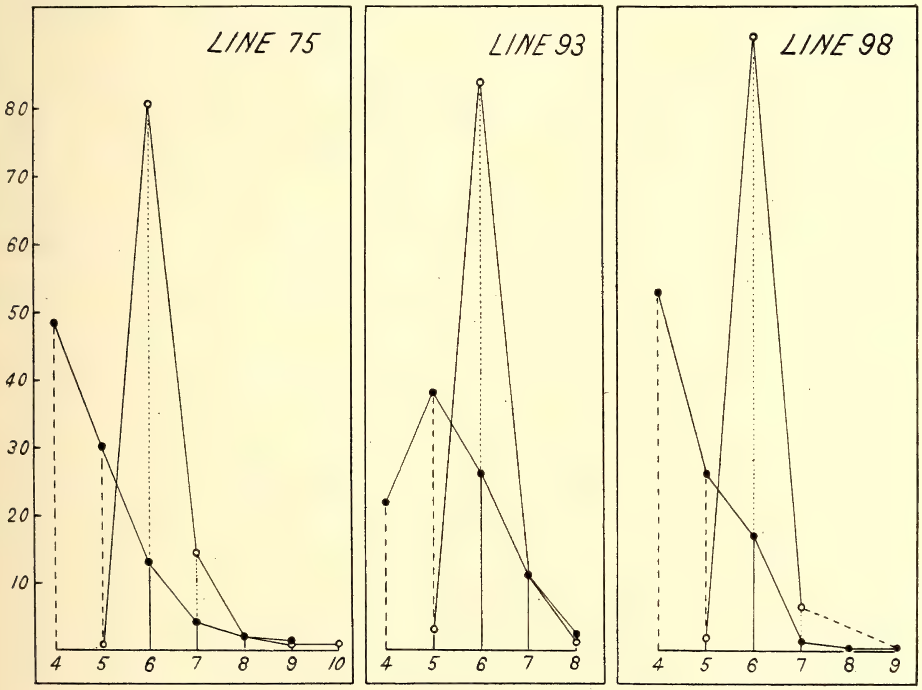


FIG. 15. Percentage frequency distribution of total bundles at base of hypocotyl. Primary double bundles counted as single bundles.

the total number of bundles at the base of the hypocotyl, it therefore becomes a question as to whether we should count each primary double bundle as a single strand or as a double strand; adding, of course, the number of intercalary bundles in each case.

The distribution of total bundle number at this level according to the former method (primary double bundles counted as one, plus intercalaries) is shown in table 7, for both dimerous and trimerous seedlings. The results are shown clearly in figure 15.[9] The modal number is on 4 (lines 75, 98, 139, and 143) or 5 (line 93) bundles in the case of the dimerous seedlings, but invariably on 6 in the trimerous plantlets of the five lines. The distribution of number of bundles is almost wholly skew in the case of the normal seedlings, line 93 being slightly different from the others, but fairly symmetrical in the trimerous series.

The constants given in table 8 show that on the average the trimerous plants have from 0.77 to 1.91 bundles more than the dimerous plants. This is an excess of from 14.4 to 46.7 percent instead of the 50 percent which one might expect if the increase in number of bundles were proportional to the number of cotyledons or primordial leaves.

TABLE 8. *Statistical constants for total number of bundles at base of hypocotyl of trimerous plants and their normal controls. Primary double bundles are counted as one bundle only*

	Mean	Standard Deviation	Coefficient of Variation
Line 75			
Trimerous (N = 142)	6.23±.03	0.601±.024	9.65± .39
Dimerous (N = 142)	4.85±.06	1.087±.044	22.41± .94
Actual difference	+1.38±.07	−0.486±.050	−12.76±1.01
Relative difference	28.45	44.71	
Line 93			
Trimerous (N = 155)	6.11±.02	0.434±.017	7.10± .27
Dimerous (N = 155)	5.34±.06	1.019±.039	19.07± .76
Actual difference	+0.77±.06	−0.585±.042	−11.97± .80
Relative difference	14.41	57.41	
Line 98			
Trimerous (N = 183)	6.06±.02	0.365±.013	6.02± .21
Dimerous (N = 183)	4.72±.05	0.909±.032	19.28± .70
Actual difference	+1.34±.05	−0.544±.035	−13.26± .73
Relative difference	28.39	59.85	
Line 139			
Trimerous (N = 106)	6.00±.02	0.307±.014	5.12± .24
Dimerous (N = 150)	4.09±.02	0.334±.013	8.15± .32
Actual difference	+1.91±.03	−0.027±.019	− 3.03± .40
Relative difference	46.70	8.08	
Line 143			
Trimerous (N = 221)	6.10±.03	0.613±.020	10.06± .33
Dimerous (N = 221)	4.38±.03	0.609±.020	13.91± .45
Actual difference	+1.72±.04	+0.004±.028	− 3.85± .56
Relative difference	39.27	0.66	

---

[9] Lines 139 and 143 are in essential agreement with 75, 93, and 98, and are not drawn.

The variability, both absolute and relative, of the number of bundles is higher in dimerous than in trimerous plants. It is conspicuously higher in lines 75, 93, and 98. Thus the standard deviations for the trimerous plants range from 0.37 to 0.60 in the three lines as compared with 0.91 to 1.09 in the dimerous controls. The relative differences show that the variability of the trimerous plants is from 45 to 60 percent less than that of the dimerous plants. In the case of line 143, however, the difference between the standard deviation of the two types of seedlings is very small—less, indeed, than the probable error of the difference. Practically the same condition is found in line 139.

The coefficients of variation show that the trimerous plants have a variability in bundle number which is from 5.1 to 10.1 percent of the mean number of bundles, whereas the dimerous controls have a variability which is from 8.2 to 22.4 percent of the average number. In lines 75, 93, and 98 the difference between the two types is much more conspicuous than in lines 139 and 143.

Since in practically all cases, however, the primary double bundles have already clearly become two strands at the point where the intercalaries appear, it probably gives us a better conception of total bundle number here to count each primary bundle as *two*, and to add thereto the number of intercalaries. The actual and the percentage distribution according to this method are shown in table 9. Lines 75, 93, and 139 are represented in

TABLE 9. *Total number of bundles at base of hypocotyl in trimerous and dimerous seedlings. Primary double bundles are counted as two*

	8	9	10	11	12	13	14	15	16	17	Total
Line 75											
Trimerous . .	—	—	2	8	109	12	10	—	1	—	142
Percent . .			1.41	5.63	76.76	8.45	7.04		0.70		
Dimerous . . .	69	30	23	8	8	4	—	—	—	—	142
Percent . .	48.59	21.13	16.20	5.63	5.63	2.82					
Line 93											
Trimerous . .	—	—	5	10	124	11	5	—	—	—	155
Percent . .			3.23	6.45	80.00	7.10	3.23				
Dimerous . . .	34	37	35	23	20	4	2	—	—	—	155
Percent . .	21.93	23.87	22.58	14.84	12.90	2.58	1.29				
Line 98											
Trimerous . .	—	1	4	6	161	10	1	—	—	—	183
Percent . .		0.55	2.19	3.28	87.98	5.46	0.55				
Dimerous . . .	97	43	29	10	2	1	—	—	—	1	183
Percent . .	53.01	23.50	15.85	5.46	1.09	0.55				0.55	
Line 139											
Trimerous . .	—	1	4	4	92	5	—	—	—	—	106
Percent . .		0.94	3.77	3.77	86.79	4.72					
Dimerous . . .	138	9	1	2	—	—	—	—	—	—	150
Percent . .	92.00	6.00	0.67	1.33							
Line 143											
Trimerous . .	2	3	15	31	134	25	5	4	1	1	221
Percent . .	0.90	1.36	6.79	14.03	60.63	11.31	2.26	1.81	0.45	0.45	
Dimerous . . .	150	55	9	5	—	1	1	—	—	—	221
Percent . .	67.87	24.89	4.07	2.26		0.45	0.45				

TABLE 10. *Statistical constants for total number of bundles at base of hypocotyl of trimerous plants and their normal controls. Primary double bundles are counted as two*

	Mean	Standard Deviation	Coefficient of Variation
Line 75			
Trimerous (N = 142)	12.17±.04	0.750±.030	6.17±.25
Dimerous (N = 142)	9.07±.08	1.351±.054	14.90±.61
Actual difference	+3.10±.08	−0.601±.062	−8.73±.66
Relative difference	34.18	44.49	
Line 93			
Trimerous (N = 155)	12.01±.03	0.627±.024	5.22±.20
Dimerous (N = 155)	9.86±.08	1.483±.057	15.04±.59
Actual difference	+2.15±.08	−0.856±.062	−9.82±.62
Relative difference	21.81	57.72	
Line 98			
Trimerous (N = 183)	11.97±.02	0.495±.018	4.14±.15
Dimerous (N = 183)	8.84±.06	1.190±.042	13.47±.48
Actual difference	+3.13±.06	−0.695±.046	−9.33±.50
Relative difference	35.41	58.40	
Line 139			
Trimerous (N = 106)	11.91±.04	0.558±.026	4.69±.22
Dimerous (N = 150)	8.11±.02	0.440±.017	5.43±.21
Actual difference	+3.80±.04	+0.118±.031	−0.74±.30
Relative difference	46.85	26.82	
Line 143			
Trimerous (N = 221)	11.90±.05	1.105±.035	9.28±.30
Dimerous (N = 221)	8.45±.04	.831±.027	9.84±.32
Actual difference	+3.45±.06	+0.274±.044	−0.56±.44
Relative difference	40.83	32.97	

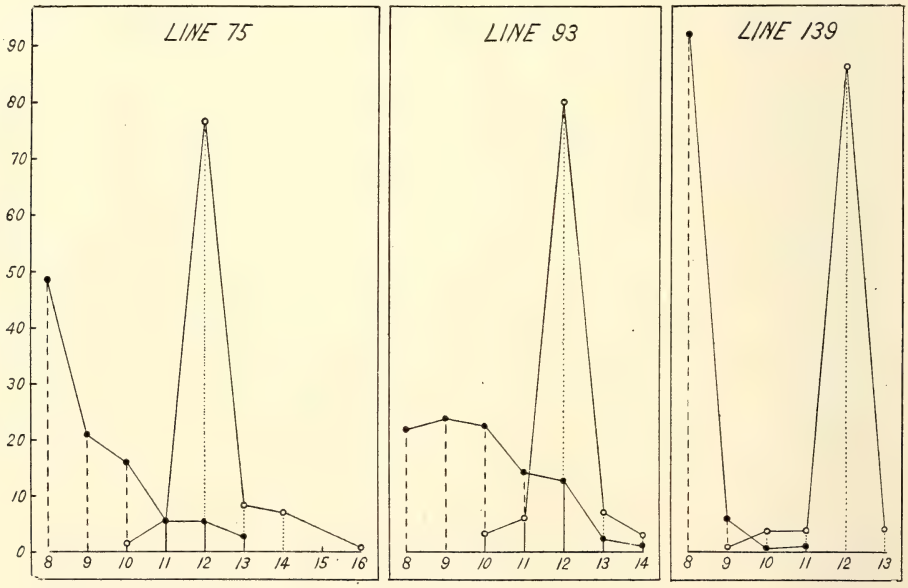


FIG. 16. Percentage frequency distribution of total bundles at base of hypocotyl in dimerous and trimerous seedlings. Primary double bundles counted as two.

figure 16. Comparison of these figures with those in figure 15 shows essentially the same type of distribution for the dimerous and trimerous plants. The grades of the classes are merely about double what they were in the former method of treatment.

The statistical constants are compared in table 10.

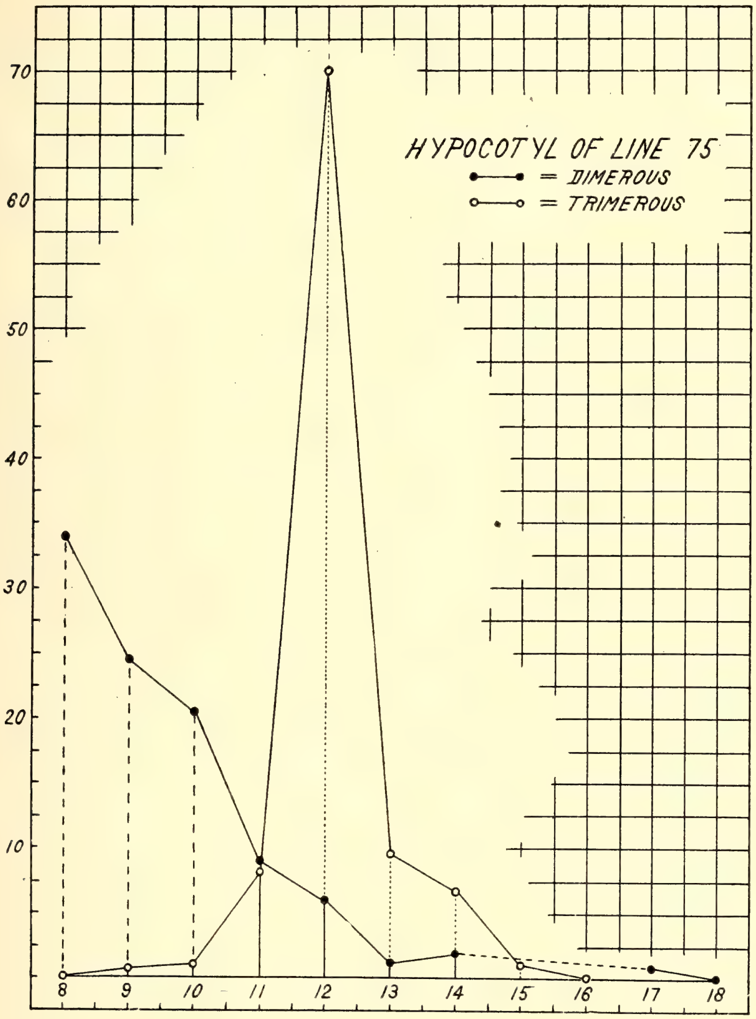


Fig. 17. Percentage frequency distribution of total bundles in central region of hypocotyl.

For all five lines the constants show a higher mean number of bundles in the trimerous than in the dimerous seedlings, the mean being approximately 12 in the former and 8 or 10 in the latter. Thus the trimerous seedlings have from 21.8 to 46.9 percent more bundles than the dimerous seedlings.

TABLE II. *Number of bundles in central region of hypocotyl of trimerous and dimerous seedlings*

	8	9	10	11	12	13	14	15	16	17	18	19	20	Total
Line 75														
Trimerous	1	3	5	36	292	40	29	5	1	4	—	—	—	416
Percent	0.24	0.72	1.20	8.65	70.19	9.62	6.97	1.20	0.24	0.96				
Dimerous	143	103	86	38	26	7	9	—	—	3	1	—	—	416
Percent	34.37	24.76	20.67	9.13	6.25	1.68	2.16			0.72	0.24			
Line 93														
Trimerous	—	—	8	32	382	82	38	12	1	—	1	—	1	557
Percent			1.44	5.75	68.58	14.72	6.82	2.15	0.18		0.18		0.18	
Dimerous	34	93	169	105	96	39	18	1	—	—	2	—	—	557
Percent	6.10	16.70	30.34	18.85	17.24	7.00	3.23	0.18			0.36			
Line 98														
Trimerous	—	1	6	12	297	21	8	—	—	—	—	—	—	345
Percent		0.29	1.74	3.48	86.09	6.09	2.32							
Dimerous	113	110	77	32	9	3	—	—	—	1	—	—	—	345
Percent	32.75	31.88	22.32	9.28	2.61	0.87				0.29				
Line 139														
Trimerous	—	—	4	8	84	6	3	1	—	—	—	—	—	106
Percent			3.77	7.55	79.25	5.66	2.83	0.94						
Dimerous	137	10	2	1	—	—	—	—	—	—	—	—	—	150
Percent	91.33	6.67	1.33	0.67										
Line 143														
Trimerous	2	1	11	14	136	21	25	6	3	1	1	—	—	221
Percent	0.90	0.45	4.98	6.33	61.54	9.50	11.31	2.71	1.36	0.45	0.45			
Dimerous	138	41	25	10	2	3	1	1	—	—	—	—	—	221
Percent	62.44	18.55	11.31	4.52	0.90	1.36	0.45	0.45						

In variability as measured by coefficient of variation, the dimerous plants exceed the trimerous throughout, conspicuously so in lines 75, 93, and 98. In their standard deviation, the dimerous also markedly exceed the trimerous in these three lines, but in lines 139 and 143 the trimerous plants slightly exceed the dimerous.

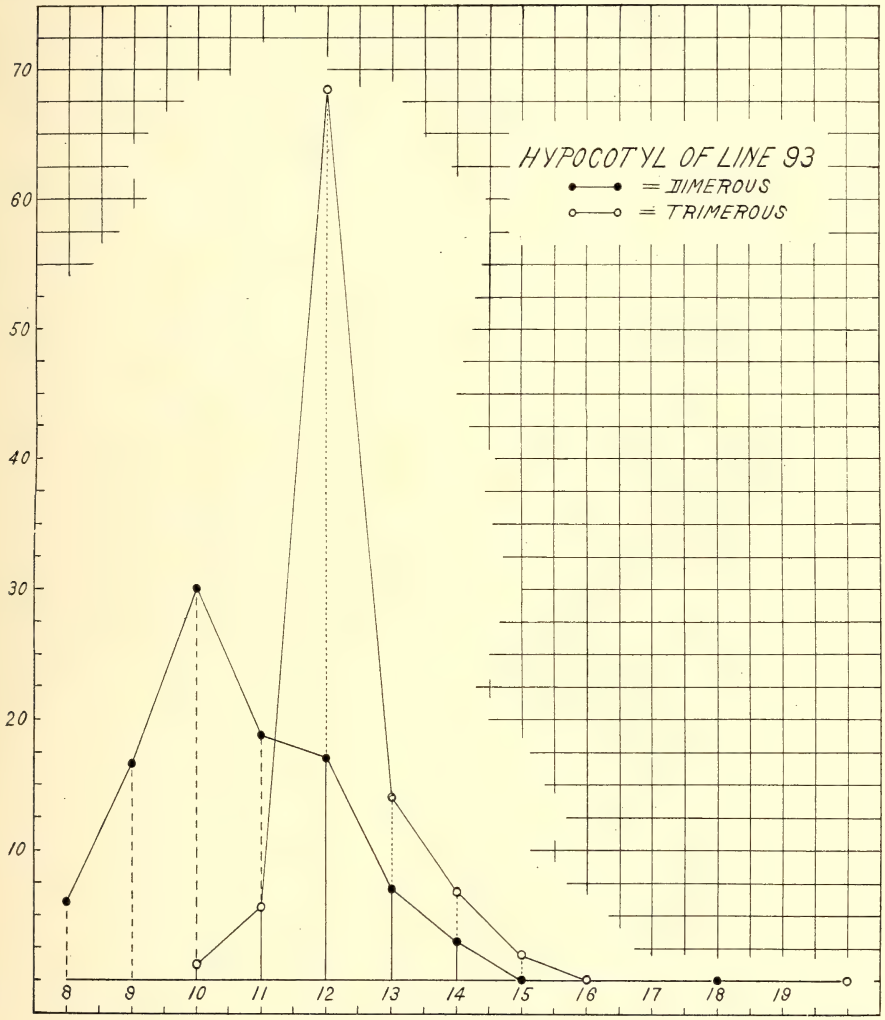


FIG. 18. Percentage frequency distribution of total bundles in central region of hypocotyl.

D. Summary for Base of Hypocotyl. For the base of the hypocotyl, therefore, it is evident that in total bundle number the trimerous seedlings decidedly exceed the dimerous ones. The intercalary bundles alone (which form but a small part of the total) tend to be more numerous in the dimerous seedlings.

In variability in bundle number at this region, dimerous seedlings in general exceed trimerous ones; although two of the five lines studied furnish slight exceptions to this rule.

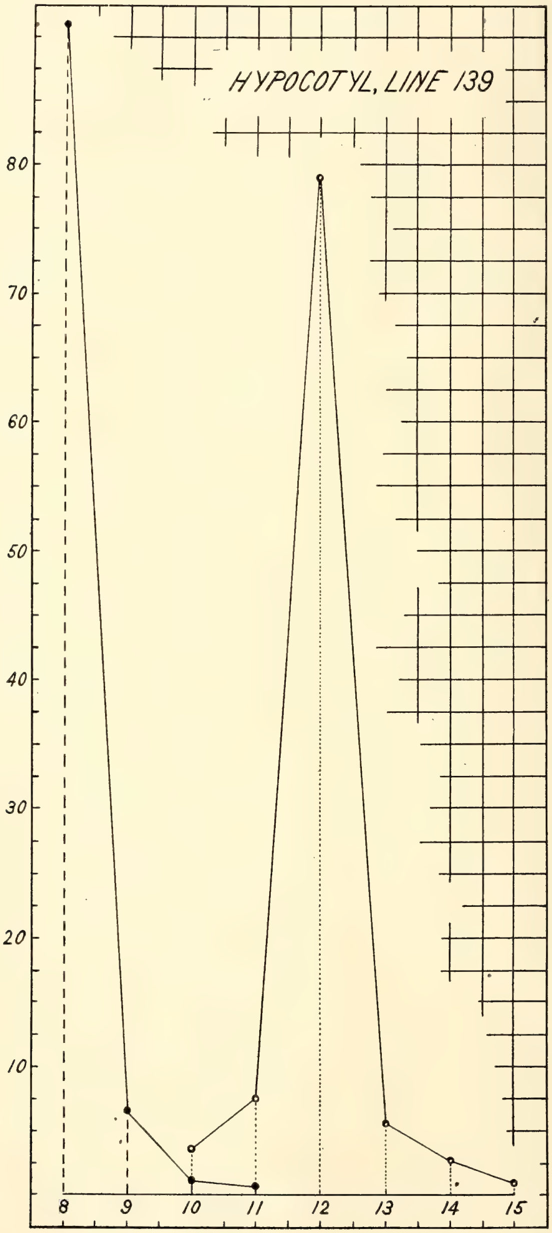


FIG. 19. Percentage frequency distribution of total bundles in central region of hypocotyl.

3. *Central Region of Hypocotyl.* In the sections made in the central regions of the hypocotyl and of the epicotyl at both Cold Spring Harbor and Storrs, the total number of bundles was counted, no distinction being made between the bundles originating from the primary double bundles and those of intercalary origin.

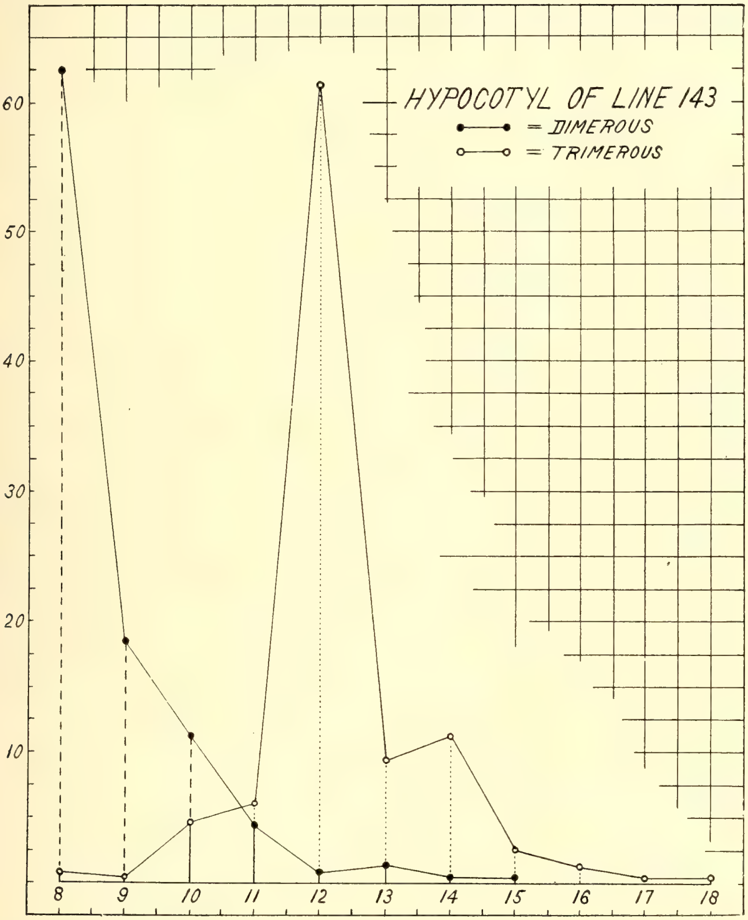


FIG. 20. Percentage frequency distribution of total bundles in central region of hypocotyl.

The frequency distributions are shown in table 11. The relative frequencies for line 75 are shown in figure 17. The form of the distributions in line 98 is in essential agreement with those in line 75 and is not represented. The distributions for line 93 are represented in figure 18. The distribution for line 139 is shown in figure 19. That for line 143 appears in figure 20.

The conspicuous feature of these distributions is the wide variation in bundle number and the conspicuous skewness of the frequencies for the normal plants of lines 75, 93, and 98. In these, bundle number ranges from 8 to 18 with a relatively large number of bundles in the lower classes. The modal number of bundles in the hypocotyl of normal seedlings of lines 75, 98, 139, and 143 is 8, while in line 93 it is 10.

The normal plants of the five lines differ conspicuously in variability. The number of seedlings falling in the modal class is relatively small and the range of variation relatively wide in lines 75, 93, and 98 as compared with line 139. Line 143 occupies an intermediate position in this regard.

In all the lines except 93 the distribution of number of bundles in the hypocotyl of normal seedlings is wholly skew, the frequency decreasing from the modal number (eight) towards the upper end of the range. In line 93 (figure 18) the distribution is also skew, but the frequency decreases from the modal number (ten) towards both ends of the range.

In the trimerous plantlets of all five series the modal number of bundles in the mid-region of the hypocotyl is 12. The extent of concentration into the modal class and the range of variation differs greatly in the five lines. This is very narrow in lines 98 and 139 and relatively wide in line 143.

The frequency distribution and figures bring out very clearly indeed the differentiation of the trimerous and dimerous seedlings in the number of vascular bundles.

TABLE 12. *Statistical constants for number of bundles in hypocotyl of trimerous and dimerous seedlings*

	Mean	Standard Deviation	Coefficient of Variation
Line 75			
Trimerous (N = 416)	12.19±.03	0.982±.023	8.06±.19
Dimerous (N = 416)	9.49±.05	1.645±.039	17.34±.42
Actual difference	+2.70±.06	−0.663±.045	−9.28±.46
Relative difference	28.45	40.30	
Line 93			
Trimerous (N = 557)	12.29±.03	0.922±.019	7.50±.15
Dimerous (N = 557)	10.62±.04	1.525±.031	14.36±.30
Actual difference	+1.67±.05	−0.603±.036	−6.86±.34
Relative difference	15.73	39.54	
Line 98			
Trimerous (N = 345)	12.03±.02	0.532±.014	4.42±.11
Dimerous (N = 345)	9.22±.04	1.197±.031	12.99±.34
Actual difference	+2.81±.04	−0.665±.034	−8.57±.36
Relative difference	30.47	55.56	
Line 139			
Trimerous (N = 106)	11.99±.05	0.694±.032	5.78±.27
Dimerous (N = 150)	8.11±.02	0.409±.016	5.04±.20
Actual difference	+3.88±.05	+0.285±.036	+0.74±.34
Relative difference	47.84	69.68	
Line 143			
Trimerous (N = 221)	12.29±.06	1.283±.041	10.44±.34
Dimerous (N = 221)	8.71±.05	1.187±.038	13.63±.45
Actual difference	+3.58±.08	+0.096±.056	−3.19±.57
Relative difference	41.10	8.09	

The differences between the lines can best be seen from the figures.

For a more critical comparison we must have recourse to statistical constants and their probable errors.

The results for the hypocotyl of trimerous seedlings and their normal controls are set forth in table 12. Without exception the number of bundles in abnormal plants is higher than that in the control plants. The differences range from 1.7 to 3.9 bundles. These differences are many times as large as their probable errors and are unquestionably significant. The relative differences are about 16 percent in line 93, 30 percent in lines 75 and 98, 41 percent in line 143, and 48 percent in line 139.

Both the standard deviation and the coefficient of variation of the number of bundles in the hypocotyl are lower in the abnormal than in the normal plants in lines 75, 93, and 98. In lines 139 and 143 the relationship of the standard deviations of the trimerous and dimerous plants is exactly reversed, that of the trimerous plants being somewhat larger than that of the dimerous series. The difference in line 143 is $+.096 \pm .056$, which is nearly twice as large as its probable error and possibly statistically significant. In line 139 the difference in standard deviation is $+.285 \pm .036$. This difference is about 8 times as large as its probable error and unquestionably significant. The percentage differences in the standard deviations in lines 75, 93, and 98 range from -40 to -56 percent. In line 143 the percentage difference is $+8$ percent, while in line 139 it is $+70$ percent.

In line 143 the coefficient of variation is higher in dimerous plants (as it is in lines 75, 93, and 98), but in line 139 the trimerous show a slightly but perhaps not significantly higher relative variability.

The results as a whole show that the difference in the variability of bundle number in the two types of seedlings in lines 139 and 143 is not the same as that in lines 75, 93, and 98.

In interpreting these results we must remember that each primary double bundle at the base of the hypocotyl almost invariably divides to form two bundles at higher levels in the hypocotyl. Occasionally one of these branches may further divide into two. It is impossible in sections made in the central region of the hypocotyl to distinguish with certainty in every case between bundles originating through a division of the original protoxylem strands and those of intercalary origin.

The simplest working assumption is that the number of bundles in the central region of the hypocotyl will be given by twice the number of primary double bundles demonstrated at the base of the hypocotyl plus the number of intercalary bundles found at the base of the hypocotyl; or the number of bundles, b, at the central region should be given by

$$b = 2p + i$$

where p = primary double bundles and i = intercalary bundles.

A comparison of the number of bundles calculated by this formula with the number actually observed in the central region of the hypocotyl may be best made in a table of double entry. Table 13 gives the results for dimerous and table 14 the results for trimerous plants of line 93. The frequencies for the cases in which the number of bundles at the mid-region of the hypocotyl calculated from the formula agrees with the number actually observed are printed in blackface type. The other lines give roughly comparable results.

TABLE 13. *Comparison of actual and theoretical number of bundles in hypocotyl of dimerous seedling*

Actual Number	8	9	10	11	12	13	14	Totals
Theoretical, $2p + i$ 8	**12**	13	6	3	—	—	—	34
9	—	**14**	17	3	1	1	1	37
10	—	1	**22**	6	5	1	—	35
11	—	—	1	**9**	9	3	1	23
12	—	—	—	1	**14**	4	1	20
13	—	—	—	—	—	**1**	3	4
14	—	—	—	—	—	—	**2**	2
Totals . .	12	28	46	22	29	10	8	155

TABLE 14. *Comparison of actual and theoretical number of bundles in hypocotyl of trimerous seedling*

Actual Number	10	11	12	13	14	15	20	Totals
Theoretical, $2p + i$ 10	**1**	1	3	—	—	—	—	5
11	—	**6**	3	—	1	—	—	10
12	—	2	**102**	12	6	1	1	124
13	—	—	—	**8**	3	—	—	11
14	—	—	—	—	**4**	1	—	5
Totals . .	1	9	108	20	14	2	1	155

It is clear that the number of hypocotyledonary bundles is not far from twice the number of primary root bundles plus the intercalary bundles. In rare cases the number of bundles in the hypocotyl is less than twice the root strands plus the number of intercalary bundles, since one of the root strands sometimes fails to divide. It may be, and not infrequently is, higher because of the appearance of extra intercalary bundles at a level higher than that sectioned at the base of the hypocotyl. In many cases the full complement of intercalary bundles has not appeared at this low level. In some cases it may be higher because of the secondary bifurcation above mentioned.

It is worth while to give the percentage frequencies of cases in which the number of bundles of the central region of the hypocotyl is given by the formula, and for comparison the relative number of cases in which it is in defect and in excess. The percentages are calculated from double entry tables like 13 and 14.

Trimerous Seedlings

	N	In Defect	$2p + i$	In Excess
Line 75	142	7.0	76.1	16.9
Line 93	155	1.3	78.1	20.7
Line 98	183	3.3	86.3	10.4
Line 139	106	7.6	80.2	12.3
Line 143	221	0.9	74.7	24.4

Dimerous Seedlings

	N	In Defect	$2p + i$	In Excess
Line 75	142	2.1	51.4	46.5
Line 93	155	1.9	47.7	50.3
Line 98	183	3.8	59.0	37.2
Line 139	150	0.7	98.7	0.7
Line 143	221	0.9	81.0	18.1

With the exception of the dimerous seedlings of line 139, the actually observed number of bundles is in excess of the number given by the formula.

In lines 75, 93, and 98 the excess is far greater in dimerous than in trimerous seedlings. Thus in the dimerous class about 40 percent of the seedlings show a number of bundles in the central region of the hypocotyl which is in excess of twice the number of primary double bundles plus the number of intercalary bundles at the base of the hypocotyl. In the case of the trimerous seedlings the excess is much smaller, being roughly 20 percent. Thus it is clear that, *especially in the normal seedlings*, a large number of the intercalary bundles do not extend to the base but appear in the axis, ending blindly below, or that a considerable proportion of the primary double bundles divide into more than two bundles.

In line 143 the number of cases in which the observed number of bundles is greater than the calculated number is much more nearly equal in the two types of seedlings. Thus in the trimerous seedlings 24.4 percent of the seedlings have a number of bundles in the central region of the hypocotyl greater than $2p + i$, whereas in the dimerous seedlings there are 18.1 percent of seedlings of this class. In line 139 only 0.7 percent of the dimerous seedlings show a number of bundles in excess of $2p + i$, whereas in the trimerous seedlings 12.3 percent are in excess.

Thus lines 139 and 143 give results diametrically opposed to those of the first three discussed.[10]

Summary for Central Region of Hypocotyl. It is evident from the above statements that the number of bundles in the hypocotyl of trimerous is decidedly higher than in that of dimerous seedlings; that in general the bundle number is more variable in dimerous than in trimerous seedlings; and that the intercalary bundles generally extend to a lower level in the hypocotyl of trimerous than in that of dimerous seedlings.

---

[10] Note that the extremely small excess in line 139 may be due to the extraordinarily normal character of the vascular system of the dimerous plants of this line.

TABLE 15. *Number of bundles in central region of epicotyl of trimerous and dimerous seedlings*

	10	11	12	13	14	15	16	17	18	19	20	21	22	Total
Line 75														
Trimerous	—	—	3	16	63	164	93	41	27	4	4	1	—	416
Percent	—	—	0.72	3.85	15.14	39.42	22.36	9.86	6.49	0.96	0.96	0.24	—	
Dimerous	1	4	336	46	16	10	3	—	—	—	—	—	—	416
Percent	0.24	0.96	80.77	11.06	3.85	2.40	0.72							
Line 93														
Trimerous	—	—	5	18	47	236	129	56	51	10	4	—	1	557
Percent	—	—	0.90	3.23	8.44	42.37	23.16	10.05	9.16	1.80	0.72	—	0.18	
Dimerous	1	6	479	42	18	10	1	—	—	—	—	—	—	557
Percent	0.18	1.08	86.00	7.54	3.23	1.80	0.18							
Line 98														
Trimerous	—	—	8	24	69	176	49	9	7	1	1	1	—	345
Percent	—	—	2.32	6.96	20.00	51.01	14.20	2.61	2.03	0.29	0.29	0.29	—	
Dimerous	—	—	316	23	4	1	1	—	—	—	—	—	—	345
Percent	—	—	91.59	6.67	1.16	0.29	0.29							
Line 139														
Trimerous	—	—	—	8	21	38	24	9	4	2	—	—	—	106
Percent	—	—	—	7.55	19.81	35.85	22.64	8.49	3.77	1.89	—	—	—	
Dimerous	—	—	131	16	3	—	—	—	—	—	—	—	—	150
Percent	—	—	87.33	10.67	2.00									
Line 143														
Trimerous	—	—	5	9	19	54	49	37	31	9	6	2	—	221
Percent	—	—	2.26	4.07	8.60	24.43	22.17	16.74	14.03	4.07	2.71	0.90	—	
Dimerous	—	—	169	34	11	5	2	—	—	—	—	—	—	221
Percent	—	—	76.47	15.38	4.98	2.26	0.90							

4. *Central Region of Epicotyl.* The frequency distributions of the number of bundles occurring in the mid-region of the epicotyl appear in table 15 for both the abnormal and the control plants. These distributions

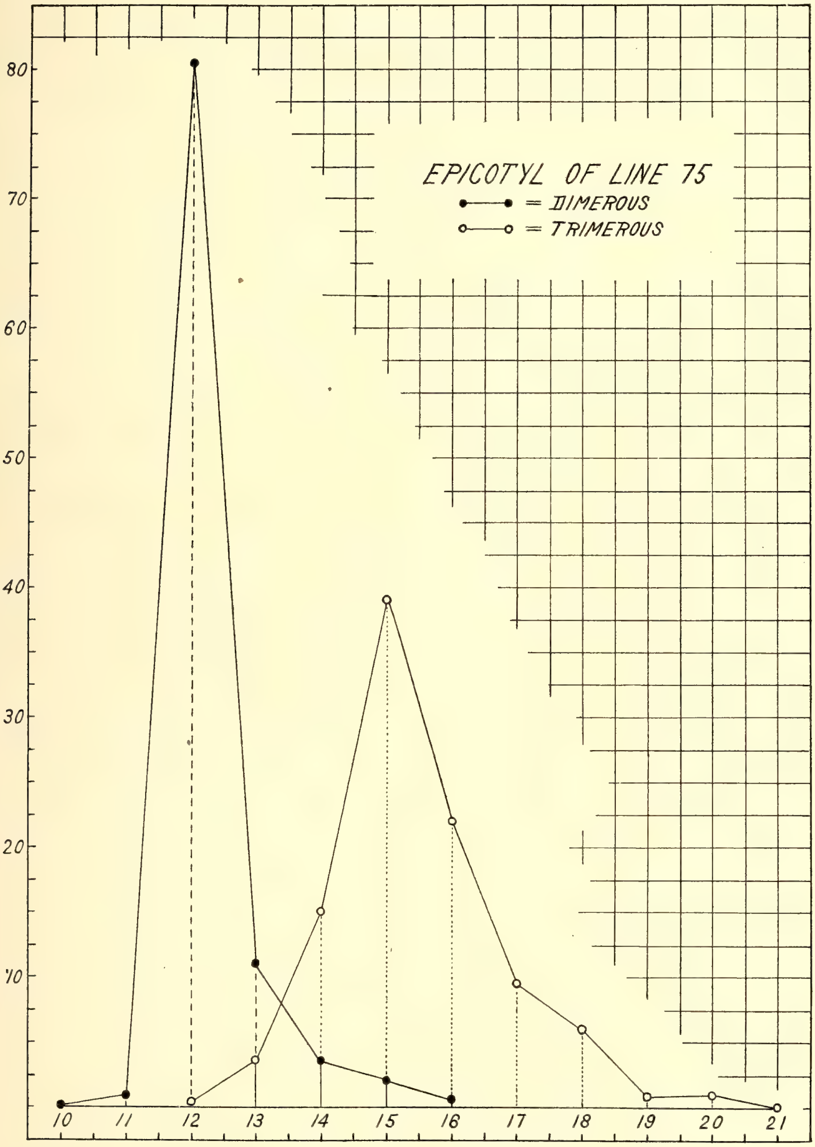


FIG. 21. Percentage frequency distribution of number of bundles in central region of epicotyl.

reduced to a percentage basis are represented graphically in figure 21 for line 75, in figure 22 for line 98, and in figure 23 for line 143. The distributions

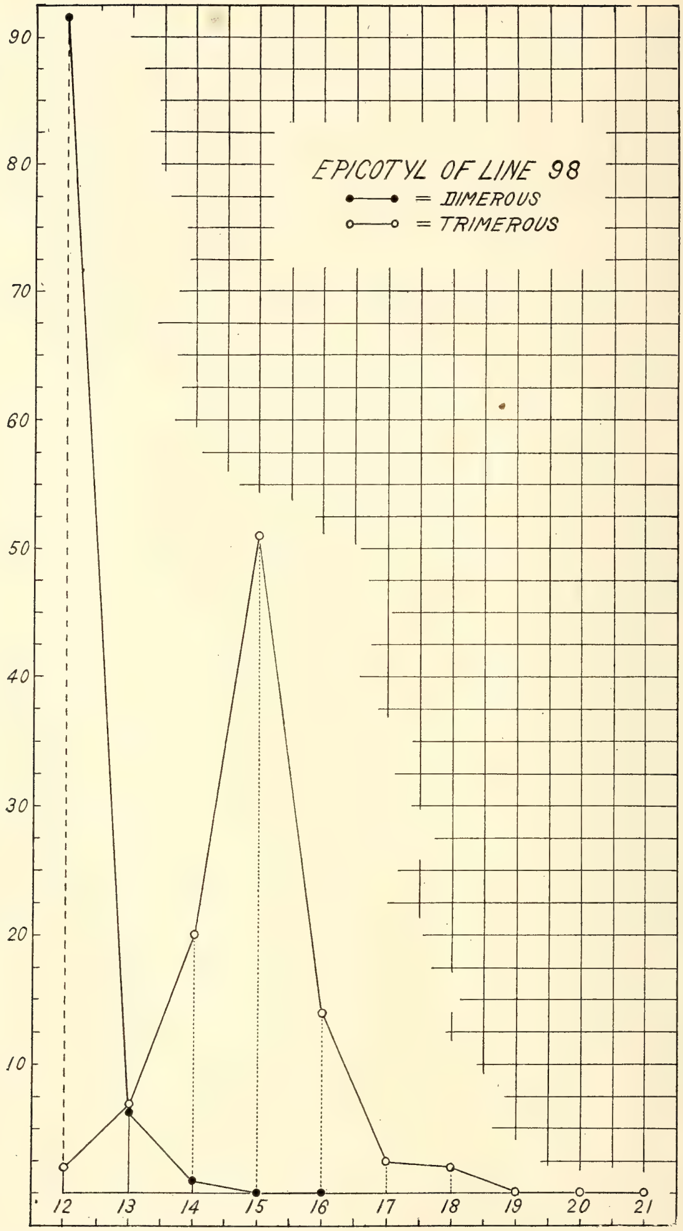


FIG. 22. Percentage frequency distribution of number of bundles in central region of epicotyl.

for line 93 are essentially the same as those for line 75. The graph for line 139 is in essential agreement with that for line 98 and is not drawn.

In the dimerous plants the difference between the form of the frequency distributions for number of epicotyledonary bundles in lines 75 and 93 on the one hand and lines 98, 139, and 143 on the other is more apparent than real. All five lines agree in showing the frequencies for the dimerous plants largely concentrated in a single modal class with a slight but evident skewness toward higher numbers of bundles. In the case of lines 75 and 93 there is a little over 1 percent of plants with fewer than the modal number of bundles, whereas in lines 98, 139, and 143 these do not occur in series of the numbers sectioned. It is quite possible that the examination of a

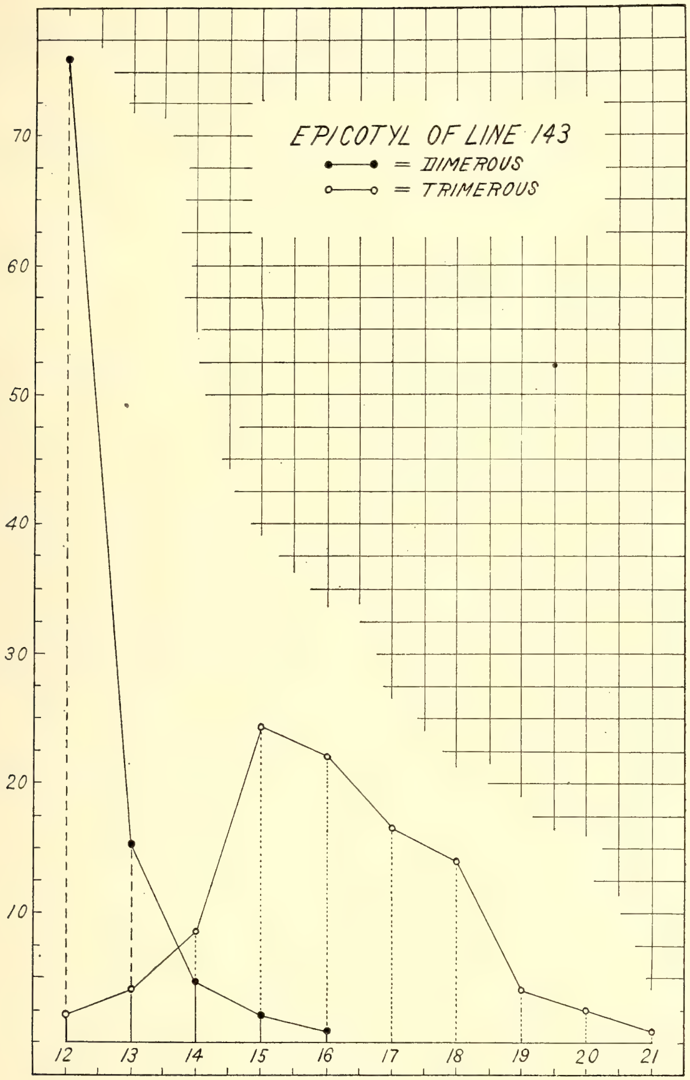


FIG. 23. Percentage frequency distribution of number of bundles in central region of epicotyl.

larger series of plantlets would result in the finding of such seedlings in lines 98, 139, and 143, thus bringing the five series into full agreement.

In the trimerous seedlings the number of bundles shows rather wide, and fairly symmetrical, distribution about the modal class, which is 15 bundles. The lines differ, however, to a considerable extent in the amount of variation from the modal class. In lines 75, 93, and 98 the frequencies are to a far greater extent concentrated into the modal class, which contains from 39 to 51 percent of the frequencies, than in line 143, which contains only 24 percent of the cases. Line 139 is intermediate between these two extremes.

For a more precise comparison we utilize the constants set forth in table 16.

TABLE 16. *Statistical constants for number of bundles in epicotyl of trimerous and dimerous seedlings*

	Mean	Standard Deviation	Coefficient of Variation
Line 75			
Trimerous (N = 416)	15.47±.04	1.355±.032	8.76±.21
Dimerous (N = 416)	12.27±.02	0.735±.017	5.99±.14
Actual difference	+3.20±.04	+0.620±.036	+2.77±.24
Relative difference	26.08	84.35	
Line 93			
Trimerous (N = 557)	15.65±.04	1.372±.028	8.77±.18
Dimerous (N = 557)	12.19±.02	0.615±.012	5.05±.10
Actual difference	+3.46±.04	+0.757±.030	+3.72±.20
Relative difference	28.38	123.09	
Line 98			
Trimerous (N = 345)	14.89±.04	1.152±.030	7.74±.20
Dimerous (N = 345)	12.11±.02	0.416±.011	3.44±.09
Actual difference	+2.78±.04	+0.736±.032	+4.30±.22
Relative difference	22.96	176.92	
Line 139			
Trimerous (N = 106)	15.24±.08	1.285±.060	8.44±.39
Dimerous (N = 150)	12.15±.02	0.406±.016	3.35±.13
Actual difference	+3.09±.08	+0.879±.062	+5.09±.41
Relative difference	25.43	216.50	
Line 143			
Trimerous (N = 221)	16.10±.08	1.750±.056	10.87±.35
Dimerous (N = 221)	12.36±.03	0.757±.024	6.13±.20
Actual difference	+3.74±.09	+0.993±.061	+4.74±.40
Relative difference	30.26	131.18	

These results show that without exception the average number of bundles in the epicotyl is higher in trimerous than in dimerous seedlings. The difference ranges from 2.8 to 3.7 bundles. The probable errors of these differences are so small that there can be no reasonable doubt of their significance. In relative terms, the number of bundles in the abnormal plant is from 23.0 to 30.3 percent higher than that in the normal plant.

The variability of bundle number, both absolute and relative, is far higher in the abnormal (trimerous) plants. The relative differences show that the trimerous plants are from 84 to 217 percent more variable than the dimerous in the number of bundles in the central region of the epicotyl.

We now have to consider the relative number of bundles in the hypocotyl and in the epicotyl of the same plant. The constants for the normal plants are shown in table 17 and for the trimerous seedlings in table 18.

TABLE 17. *Comparison of statistical constants for number of bundles in hypocotyl and epicotyl of same plant. Seedlings with two cotyledons and two primordial leaves*

	Mean	Standard Deviation	Coefficient of Variation
Line 75 (N = 416)			
Hypocotyl	9.49±.05	1.645±.039	17.34±.42
Epicotyl	12.27±.02	0.735±.017	5.99±.14
Actual difference	+2.78±.05	−0.910±.043	−11.35±.44
Relative difference	29.29	55.31	
Line 93 (N = 557)			
Hypocotyl	10.62±.04	1.525±.031	14.36±.30
Epicotyl	12.19±.02	0.615±.012	5.05±.10
Actual difference	+1.57±.04	−0.910±.033	− 9.31±.32
Relative difference	14.78	59.67	
Line 98 (N = 345)			
Hypocotyl	9.22±.04	1.197±.031	12.99±.34
Epicotyl	12.11±.02	0.416±.011	3.44±.09
Actual difference	+2.89±.04	−0.781±.033	− 9.55±.35
Relative difference	31.34	65.24	
Line 139 (N = 150)			
Hypocotyl	8.11±.02	0.409±.016	5.04±.20
Epicotyl	12.15±.02	0.406±.016	3.35±.13
Actual difference	+4.04±.03	−0.003±.023	− 1.69±.24
Relative difference	49.82	0.73	
Line 143 (N = 221)			
Hypocotyl	8.71±.05	1.187±.038	13.63±.45
Epicotyl	12.36±.03	0.757±.024	6.13±.20
Actual difference	+3.65±.06	−0.430±.045	− 7.50±.49
Relative difference	41.91	36.23	

Normal and abnormal plants have in common a larger number of bundles in the epicotyl. The differences between the means for the two organs are clearly significant in comparison with their probable errors. The percentage differences show that the epicotyl has from 15 to 50 percent more bundles than the hypocotyl.

In the dimerous seedlings the variabilities, both absolute and relative, as measured by the standard deviation and coefficient of variation, are consistent in indicating a higher variability of bundle number in the hypocotyl. The difference is, however, very slight in line 139.

The difference between the variability of the hypocotyl and that of the epicotyl in the normal seedling as measured in terms of the standard deviation is from 0.8 to 0.9 bundle, or from 55 to 65 percent of the larger value in lines 75, 93, and 98. In line 143 the difference is only 0.4 bundle, or 36 percent. In line 139 there is practically no difference in the standard deviation of bundle number in the mid-region of the first two internodes of the seedling.

TABLE 18. *Comparison of statistical constants for number of bundles in hypocotyl and epicotyl of same plant. Seedlings with three cotyledons and three primordial leaves*

	Mean	Standard Deviation	Coefficient of Variation
Line 75 (N = 416)			
Hypocotyl	12.19±.03	0.982±.023	8.06±.19
Epicotyl	15.47±.04	1.355±.032	8.76±.21
Actual difference	+3.28±.05	+0.373±.040	+0.70±.28
Relative difference	26.90	37.98	
Line 93 (N = 557)			
Hypocotyl	12.29±.03	0.922±.019	7.50±.15
Epicotyl	15.65±.04	1.372±.028	8.77±.18
Actual difference	+3.36±.05	+0.450±.033	+1.27±.22
Relative difference	27.33	48.81	
Line 98 (N = 345)			
Hypocotyl	12.03±.02	0.532±.014	4.42±.11
Epicotyl	14.89±.04	1.152±.030	7.74±.20
Actual difference	+2.86±.04	+0.620±.033	+3.32±.22
Relative difference	23.77	116.54	
Line 139 (N = 106)			
Hypocotyl	11.99±.05	0.694±.032	5.78±.27
Epicotyl	15.24±.08	1.285±.060	8.44±.39
Actual difference	+3.25±.09	+0.591±.068	+2.66±.48
Relative difference	27.11	85.16	
Line 143 (N = 221)			
Hypocotyl	12.29±.06	1.283±.041	10.44±.34
Epicotyl	16.10±.08	1.750±.056	10.87±.35
Actual difference	+3.81±.10	+0.467±.069	+0.43±.49
Relative difference	31.00	36.40	

Basing the comparisons on the coefficient of variation, we note that the coefficients for the hypocotyl range from 13.0 to 17.3 percent, whereas those for the epicotyl range from 3.4 to 6.0 percent in lines 75, 93, and 98. Thus there is a difference of about 10 percent in the coefficient of variation of bundle number in the hypocotyl and epicotyl (of the normal seedlings) of these lines. In line 143 this difference is only −7.50 percent. In line 139 it is only −1.69 percent.

The statistical relationship is in full accord with the anatomical findings recorded above (p. 68) where it was shown that the intercalary bundles of the hypocotyl as they approach the cotyledonary node fuse with the (normally 8) bundles originating by the division of the (normally 4) protoxylem poles of the primary root and completely lose their individuality, exactly six bundles emerging from the complex irrespective of the number which have entered it from the hypocotyl.[11] Immediately above the cotyledons the six remaining bundles approach, closing the cotyledonary gaps and forming a ring, the six members of which almost immediately divide, giving rise to the modal number, 12, which persists throughout the length of the epicotyl. It is apparently the disappearance of the intercalary bundles as a conspicuous feature of the topography which results in the lowered variability of bundle number in the epicotyl as compared with the hypocotyl.

If this conclusion be true, we should find the least difference in the variability of number of bundles in the central regions of the first two internodes in the lines in which intercalary bundles are least conspicuous as a feature of the vascular topography. As a matter of fact, this condition is strongly supported by the results for the five lines investigated. Turning back to table 6, showing the constants for number of intercalary bundles, we note that lines 75, 93, and 98 have on the average from 0.60 to 0.83 intercalary bundle per (normal) plant. These are the lines showing a relative difference of 55 to 65 percent in the standard deviations as compared with 36 percent in line 143 with an average of 0.31 intercalary bundle, and of only 0.73 percent for line 139 which has an average of only 0.07 intercalary bundle per plant. The differences in the coefficients of variation for hypocotyl and epicotyl are from −9.3 to −11.4 percent in the three lines with from 0.6 to 0.8 intercalary bundle per plant, −7.5 percent in line 143 with an average of 0.31 intercalary bundle, and only −1.7 percent in line 139 with an average of only 0.07 intercalary bundle.

In the trimerous seedlings the relationship between the variation of the number of bundles in the hypocotyl and in the epicotyl is *just the reverse* of that found in the normal type. Variability as measured by the standard deviation is significantly higher in the epicotyl of all lines studied. The same is true if the coefficient of variation be used as a measure of variability, although the differences for lines 75 and 143 are not large.

The anatomical explanation of this fact seems to be found in the peculiarities of behavior at the cotyledonary node. As pointed out above (p. 70), the epicotyledonary ring is typically made up of nine strands instead of the six characteristic of the normal plant. There is, therefore, in the modal case an increase of fifty percent in the number of bundles in the epicotyledonary ring of the trimerous plant as compared with the dimerous plant. Many of these bundles, but not all, divide to form the bundle system characteristic of the main course of the epicotyl. It is this variability in the extent of division of the bundles of the epicotyledonary ring which, in connection with the low variability of the hypocotyl due to the formation of but few intercalary bundles (except in lines 139 and 143, where the number is about the same in normal and abnormal seedlings), brings about the great variability in the bundle number of the mid-region of the epicotyl as compared with the mid-region of the hypocotyl, in the trimerous plants.

---

[11] This statement is based on a more detailed anatomical study of a portion of the seedlings.

This condition furnishes an excellent example of the importance of a knowledge of descriptive morphology as an aid in interpreting biometric constants.

COMPARISON OF BUNDLE NUMBER IN THE FIVE LINES STUDIED

From the genetic standpoint it seems a matter of considerable interest to determine whether the three nominally pure lines[12] are differentiated with respect to their vascular anatomy.

A comparison of the percentage frequency distributions and the figures of the foregoing discussion will convince the reader that certain of the lines may be differentiated either in mean number of bundles, or in variability of number of bundles, or in both average number and variability of bundle number.

Since we hope to return to this problem later with even more extensive data, it seems unnecessary to consider the differences in the distributions and constants in detail at this time.

The results of this brief and superficial comparison seem to indicate that while different lines may not differ greatly in respect to certain of their vascular characters they may be differentiated with respect to others.

SUMMARY

This paper presents the results of a comparative and biometric study of the gross vascular anatomy of the seedling of *Phaseolus vulgaris*.

Two morphological types are considered: the normal, or dimerous, seedling with two cotyledons and two primordial leaves, and the trimerous seedling with three cotyledons and three primordial leaves.

In normal seedlings, the vascular system of the root is typically tetrarch (with four protoxylem poles), and gives rise in the base of the hypocotyl to eight bundles which continue to the cotyledonary node. From the vascular complex at this point two strands are given off to each cotyledon and six are left, each of which divides into two to produce the typical twelve-bundled condition of the epicotyl.

The trimerous seedlings typically possess six root poles instead of four, twelve bundles in the hypocotyl instead of eight, and nine primary epicotyledonary bundles instead of six. The nine primary epicotyledonary bundles may not all divide, however, so that the number of bundles in the central region of the epicotyl is variable, ranging in general from fourteen to eighteen.

---

[12] While the material employed in this study traces its origin from individual plants, the possibility of hybridization in the field is not excluded. Thus any comparison which may be made in this place must be regarded as preliminary merely.

In both types of seedlings, but more frequently in the normal ones, additional or intercalary bundles appear in the hypocotyl, either *de novo* or as a result of division of the primary strands.

The following constants[13] (table 19) for bundle number (at the different levels studied) epitomize the differences which characterize the two types of seedlings.

TABLE 19

	Trimerous Seedlings			Dimerous Seedlings		
	Mean	S. D	C. V.	Mean	S. D.	C. V.
Root poles						
Minimum	5.02	.654	13.02	4.01	.081	2.03
Maximum	5.16	.739	14.47	4.13	.338	8.18
Mean	5.09	.707	13.87	4.05	.171	4.19
Primary double bundles						
Minimum	5.81	.288	4.86	4.02	.140	3.48
Maximum	5.98	.581	10.01	4.52	.666	14.74
Mean	5.91	.405	6.87	4.19	.411	9.66
Intercalary bundles						
Minimum	.09	.292	156.62	.07	.261	105.79
Maximum	.29	.686	381.67	.83	1.024	355.48
Mean	.19	.491	274.92	.49	.687	182.70
Mid-region of hypocotyl						
Minimum	11.99	.532	4.42	8.11	.409	5.04
Maximum	12.29	1.283	10.44	10.62	1.645	17.34
Mean	12.16	.883	7.24	9.23	1.193	12.67
Mid-region of epicotyl						
Minimum	14.89	1.152	7.74	12.11	.406	3.35
Maximum	16.10	1.750	10.87	12.36	.757	6.13
Mean	15.47	1.383	8.92	12.22	.586	4.79

The variability of root pole number is distinctly higher in trimerous than in dimerous seedlings, because of the fact that in *all* seedlings a four-poled condition is characteristic of the main root system and prevails even in the trimerous forms up to within a few millimeters of the base of the hypocotyl. Sections in the upper root region in such seedlings therefore show a considerable number of four- and five-bundled individuals.

The number of intercalary bundles is highly variable in both seedling types. The standard deviation is distinctly larger in the dimerous forms, but because of the generally lower average number of intercalary bundles in trimerous seedlings, the relative variabilities as measured by the coefficient of variation are higher in the trimerous type.

In the central region of the hypocotyl the variability of bundle number, both absolute and relative, is far higher in the dimerous seedlings, due in large part to the generally higher standard deviation of the number of intercalary bundles in the dimerous type.

In the central region of the epicotyl just the reverse is true, the variability of bundle number being higher in the trimerous than in the dimerous seedling. This is evidently due to the facts (*a*) that the intercalary bundles of the hypocotyl are quite lost in the cotyledonary nodal vascular complex, and thus do not affect the variability of the dimerous plants; and (*b*) that the doubling of the primary epicotyledonary bundles which almost invariably occurs in the normal seedling may not always take place, at least not at as low a level as the central region of the epicotyl, in the abnormal type.

---

[13] Data for number of root poles are available for only three of the five lines.

Conclusions

The results of the foregoing morphological and biometric analyses justify the emphasis at this point of certain general considerations.

1. External differentiation such as that which characterizes dimerous and trimerous seedlings of *Phaseolus vulgaris* is accompanied by profound differences in internal structure.

2. Anatomical characters are by no means constant. On the contrary, they are very variable even in series of individuals which are genetically highly homogeneous. Morphological investigations based on limited series of individuals may, therefore, result in inadequate conceptions.

3. Variation in anatomical structure is not constant for the plant as a whole, but may differ from region to region or from organ to organ. Thus in the regions of the seedling here under consideration, hypocotyl and epicotyl differ widely in the variability of bundle number. Furthermore, differences in variability from organ to organ or from region to region are not constant, but may be conditioned by other morphological features. To illustrate from the case in hand, the variability of bundle number of normal seedlings is higher in the hypocotyl than in the epicotyl. In seedlings with three cotyledons and three primordial leaves, just the reverse is true. These differences in biometric constants are readily understandable in the light of a knowledge of comparative morphology.

4. The results of this study emphasize the importance of the use of both biometric and comparative methods to supplement each other in any attack upon the problems of general morphology or of morphogenesis.

ASPERGILLUS FLAVUS, A. ORYZAE, AND ASSOCIATED SPECIES

Charles Thom and Margaret B. Church

(Received for publication August 30, 1920)

Cultures of fermented food products of the Orient made from rice, other cereals, and soy beans show a number of characteristic types of Aspergillus. Some of these are manifestly only contaminations. A few of them are so closely identified with these food products as to call for comparative study to determine their significance in the fermentation processes under investigation. These organisms are recorded under the names *A. flavus* Link, *A. oryzae* Ahlb., *A. Wentii* Wehmer, and *A. tamari* Kita. Study of cultures from many correspondents and from a wide variety of foodstuffs shows clearly that these forms are not limited to the Oriental fermentation industries but are cosmopolitan. The numerous strains found align themselves into groups of closely related forms which may for convenience be considered here under three series names, *Aspergillus flavus-oryzae*, *A. Wentii*, and *A. tamari*.

Aspergillus flavus-oryzae Series

The saké industry of Japan is based upon the diastatic power of *Aspergillus oryzae* (Ahlb.) Cohn.[1] The organism as actually used is a well-marked form and its activities have been extensively discussed. Cultures of this species which have been distributed widely from the Centralstelle[2] at Amsterdam show the morphology and culture reactions clearly described by Wehmer[3]. Among large numbers of mold cultures from many sources only one culture which might be confused with the saké organism has been received from a source unconnected with the Oriental fermentation industries.

When, however, numerous cultures from the soy or shoyu industry of Japan and China are brought together, a whole series of forms are found which bridge the gap morphologically between *A. oryzae* as the saké organism and *A. flavus* as described and distributed also by Wehmer (*loc. cit.*, p. 81).

---

[1] Cohn, F. Ueber Schimmelpilze als Gährungserreger (*A. oryzae*). Jahresb. Schlesisch. Gesellsch. f. vaterland. Cultur **61** (1883): 226–229. Breslau, 1884.

[2] Centralstelle für Pilzkulturen, Roemer Visscherstraat 1, Amsterdam.

[3] Wehmer, C. Die Pilzgattung Aspergillus in morphologischer, physiologischer, und systematischer Beziehung unter besonderer Berücksichtigung der mitteleuropaeischen Species. Mém. Soc. Phys. Hist. Nat. Genève **33**^{2}: 1–156. Pls. 1–5. 1901. This paper is commonly cited as Wehmer, Monograph (Monogr.).

Material taken directly from fermenting vats in China by Dr. Yamei Kin, formerly of the Bureau of Chemistry, shows strains of this character. Inoculating material furnished by Dr. Teizo Takahashi for experimental work on the fermentation of soy sauce or shoyu proved to be a member of this series. Dr. Takahashi had selected his strain for this type of fermentation from among several recognized and studied by him in Tokyo. Our thanks are due to Dr. Takahashi for discussing his views upon the group of organisms used by the fermentation industries of his country. These forms showed variations toward the saké organism and others markedly of the "*flavus*" type. All of these strains are regarded by him as varieties of *Aspergillus oryzae*, not *A. flavus*. In the fermenting samples examined, the dominant organism in every case has been nearer *A. flavus* in the sense of Brefeld and Wehmer than *A. oryzae* (Ahlb.) Cohn. The same condition is readily disclosed by cultures from certain of the koji products distributed under the patents[4] of Takamine in which the name *A. oryzae* is used, not *A. flavus*. Although the study of strains widely separated in the series gives easily measurable differences, comparison of large numbers of strains from many sources furnishes intermediate forms which break down the value of such contrasting characters. All of these forms show mixtures of yellow and green color when grown on Czapek's solution agar which, when compared with Ridgway's plates, are found to be closely related. The whole group is found to possess conidiophore stalks and conidia with walls pitted. Stalk walls when examined with low magnification are often recorded as delicately rough, and conidia as delicately rough or spiny. Careful examination with high powers shows these appearances to be due to pits. Upon the ripe conidia the pits, instead of being circular, are commonly elliptical, giving an appearance sometimes designated as areolate.

Variations in length and diameter of stalk, thickness of wall, and number and arrangement of sterigmata are found, but the texture and markings of the walls, the formation, shape, and development of parts appear to link together related forms, hence to have value in characterization. Accuracy in these observations becomes, therefore, essential. Johnston[5] notes discrepancies in the description of the same culture by different workers. We find the same difficulty in our own notes. Cell walls examined with the lower powers, especially the dry objectives, may be recorded as rough or spinulose. The same cell walls examined with the apochromatic objective appear pitted. It has been found necessary to make many examinations of each species or strain studied, both separately and in comparison with other related forms.

The data used in this paper have been obtained by using a Zeiss 3 mm., N. A. 1.30 apochromatic objective with a Zeiss 12 x compensation ocular.

---

[4] Takamine, J., U. S. nos. 27401; 525.820; 525.823; 525.824.

[5] Johnston, J. R. The entomogenous fungi of Porto Rico. Bull. Board Commrs. Agr. Porto Rico **10**: 17. 1915.

The forms reported here have each been restudied several times, some of them at intervals of several years, to determine which characters were variable with conditions of culture and which were stable.

The following cultural descriptions of *A. flavus*, *A. oryzae*, *A. parasiticus*, and *A. effusus* are prepared as typical for races or groups of nearly related strains, which represent fairly widely separated portions of the whole series.

Characterization of A. flavus Link.[6] Colonies on Czapek's solution agar with sucrose, spreading widely, with floccosity limited to scanty growth of a few aerial hyphae in older and dryer areas among the erect crowded conidiophores; sclerotia at first white, then brown, hard, parenchymatous, in a few strains white-tipped, produced abundantly by some strains, scantily by others under undefined conditions, not or rarely by still others; perithecia not found. Conidial areas ranging in color in different races from *sea-foam yellow* through *chartreuse yellow*, *citron green* or *lime green*, to *mignonette green*, *Krönberg's green*, or more rarely to *ivy green* (see Ridgway XXXI. 25″ f,d,b,i,k,m;[7] approximately C. D. C. 270, 271, 266, 252, 253, 257),[8] persistent or changing in very old colonies toward *Isabella color* to *brownish olive* (Ridgway XXX. 19″ l,m), zinc orange (Ridgway XV. 13′), or even *Saccardo's umber* (Ridgway XXIX. 17″ k); reverse (or under side) and agar either uncolored or more or less intensely yellowed, from *pinkish buff*, *cinnamon buff*, to *clay* or even *Saccardo's umber* (Ridgway XXIX. 17″ d, b, to k), or in some cases even darker brown in old and dry cultures. Stalks arising separately from substratum, 400 to 700 μ ,even to 1000 μ long, 5 to 15 μ in diameter, broadening upward, with walls colorless, so pitted as to appear rough or spiny with low magnification, occasionally granuliferous, varying in thickness, gradually enlarging to form a vesicle 10 to 30 or even 40 μ in diameter. Heads in every colony varying from small with a few chains of conidia to very large stellate or columnar masses, or both mixed in the same area (fig. 1, *c* and *d*); small heads with small dome-like vesicles and a single series of a few sterigmata up to 10 to 15 μ by 3 to 5 μ; larger heads partly with simple sterigmata, partly with branched or double series, or with both in the same head; primary sterigmata 7 to 10 μ by 3 to 4 μ; secondary series 7 to 10 μ by 2.5 to 3.5 μ; conidia pyriform to almost globose, from almost colorless to yellow-green, with walls so thickened as to leave circular, elongated, or winding pits, giving a rough or echinulate effect[9] when viewed with low magnification, varying in size in different strains and even in the same culture, frequently 2 by 3 μ, 3 by 4 μ, 4 by 5 μ, or 5 by 6 μ in diameter, or even larger in some strains.

Colonies grow best in starch- and sugar-containing media; some strains fruit at temperatures up to 50° C. Spores survived heating[10] to 57.2° C. for 30 minutes and dry heat at 110° C. for 30 minutes.

This description was originally based upon culture no. 108 received from Amsterdam and identical with no. 3526 obtained directly from Wehmer.

---

[6] This characterization is revised and extended from the form furnished to Dr. John R. Johnston and published by him (*loc. cit.*).

[7] Ridgway, R. Color standards and color nomenclature. Washington, D. C., 1912.

[8] Klincksieck, P., and Valette, T. Code de couleurs. Paris, 1908.

[9] "Areolate" of Johnston.

[10] Thom, C., and Ayers, S. H. Jour. Agr. Res. **6**: 153. 1916.

This organism in Czapek's solution agar is *Krönberg's green* without color in the substratum and without sclerotia. The following supplementary cultures are cited: no. 3557.9 from corn (isolated by Clawson) differs by the production of sclerotia, and yellow color in the substratum; no. 128, after many transfers identifiable with no. 108, produced a sclerotium—former resembling no. 3557.9; no. 2773 from Demerara is *mignonette green*, produces abundant sclerotia and yellow color in reverse. In general, sclerotium formation is found correlated with the production of yellow color in the submerged mycelium and with reduced intensity of green in the conidial area.

Numerous strains with the same cultural characters have been obtained from many sources. The considerations leading to the retention of the name *A. flavus* for these forms are discussed later under "nomenclature."

A. ORYZAE SERIES

Aspergillus oryzae has been generally accepted as a valid species. It is characterized as a group of varieties by Costantin and Lucet.[11] In the typical form represented by the cultures and descriptions of Wehmer, the species is readily separated from cosmopolitan forms of *A. flavus*. In the Oriental industries in which it has been long used, the separateness of this form is largely lost. It becomes, therefore, a gigantic race in a group in which other members possess the same habits, the same essentials of structure, but differ slightly in color and greatly in size. Growth upon different substrata produces great differences in the appearance of colonies. The fruiting stalks on Czapek's solution agar are commonly 2 to 3 mm. in length, much longer on richly organic media, and are reported by Takahashi[12] to attain a length of 20 to 30 mm. upon special rice media upon which the stalks of *A. flavus* reach a length of 5 m to 8 m.

In contrast with *A. flavus* as already described, the following characterization from cultures is proposed.

A. oryzae (Ahlb.) Cohn. Colonies on Czapek's solution agar spreading broadly, pale greenish yellow (at its greenest about *lime green* to *mignonette green*. Ridgway, *loc. cit.* XXXI. 25″ YG–Y), with the green fading early to leave yellowish brown shades; sclerotia dark, produced occasionally, few and in clumps; mycelium and agar uncolored; stalks 2 to several millimeters long, up to 20 to 25 μ in diameter; heads both large and small in the same culture, predominantly large, globose, and radiate rather than calyptrate; sterigmata most commonly 1-series, occasionally 2-series, primary sterigmata up to 8 to 12 by 5 μ, secondary when present 7 to 10 by 3 μ; conidia pyriform, colorless to very slightly yellow with walls so thickened as to leave circular, elongated, or winding pits[13] giving a rough or echinulate effect when viewed with low magnification, varying in size, predominantly larger than *A. flavus*, 3 by 4 μ, 4 by 5 μ, 5 by 6 μ, 6 by 7 μ, occasionally 5 to 6 by 8 to 10 μ.

---

[11] Costantin, J., and Lucet, L. Recherches sur quelques Aspergillus pathogènes. Ann. Sci. Nat. Bot. IX, 2: 119–171. 1905.

[12] Takahashi, T. Preliminary note on the varieties of *Aspergillus oryzae*. Jour. Coll. Agr. Tokyo 1: 137–140. 1909.

[13] "Areolate" of Johnston, *loc. cit.*

This description is primarily based upon culture no. 113 from Amsterdam. The same form has been isolated at various times from fermented products (once from a Brazil nut), and received in exchange from various workers. A series of 3 varieties were first studied by Takahashi[14] in 1908. This work was continued with the accumulation of a series of strains under this name which have been furnished to us for study. These are lettered[15] with the alphabet from A to P, then skip to Z, and all are regarded as *A. oryzae.*

These cultures were transferred and grown under conditions as uniform as possible in Czapek's solution agar. The resulting colonies were arranged into a series to correspond with our conception of the relationships involved. This may be tabulated as follows:

Takahashi strains arranged in order of appearance of colonies:

H. White, nearly sterile, floccose mycelium.
O. Slight fruiting, predominantly yellow.
B. Increase of fruiting, still a floccose colony. Near *A. giganto-sulphureus.*
G. Further development of fruit at expense of floccosity.
Z. Long stalks, large heads, floccose effect.
D. Mycelium and long-stalked fruits, both evident.
N. Abundant stalks and heads, no green color. Near *A. perniciosus.*
F. Close resemblance to no. 113, *A. oryzae* of Wehmer.
I. Short-stalked, form otherwise near no. 113.
A. Still shorter.
M. Same morphology, green color more prominent.
L. Slightly paler form with shorter stalks.
C. Close to no. 108, *A. flavus* of Brefeld and Wehmer.
P. Shorter stalks (crowded; more slender type), green passing to reddish brown. Near *A. micro-virido-citrinus.*
J.
K. Aberrant forms suggesting the same line of transformation from strain C as is found in *A. effusus*, though differing from previously examined representatives.
E.

Similarly, Z, G, B, O, and H are progressive reductions from the *A. oryzae* type found in strain F.

This table shows strain F to represent approximately the form already described as *A. oryzae* (no. 113). With almost entire loss of green color and progressively increasing floccosity, strains N, D, G, B, O, and H end at H in almost complete loss of conidium production. The absence of all green color makes strain N conspicuous as a variation which might easily be regarded as a distinct species. From F the strains I, A, M, and L grade in appearance toward C, which is closely similar to *A. flavus* as already described (no. 108).

---

[14] *Loc. cit.*

[15] The lettering is maintained to correspond with Dr. Takahashi's usage in his own papers. Takahashi, T., and Yamamoto, T. On the physiological differences of the varieties of *Aspergillus oryzae* employed in the three main industries in Japan, namely saké, shóyu, and tamari manufacture. Jour. Coll. Agr. Tokyo **5**: 153–161. 1913.

The tendency toward floccosity and toward quick disappearance of green color appears again in L.

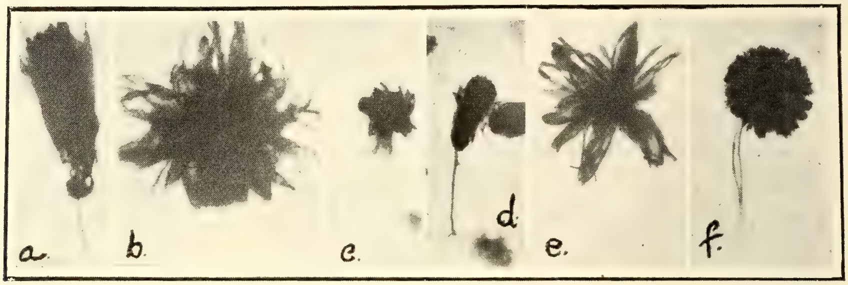


FIG. 1. The photomicrographs composing this figure represent the wide variety of heads in a species and in a strain. The magnifications are various and are not given. *a.* Calyptrate head of *A. tamari.* *b.* Radiate and large head of *A. tamari*; the same strain as *e.* *c.* Head of no. 108, type of *A. flavus.* *d.* Columnar head of no. 129; a delicate, pale form of *A. flavus.* *e.* Radiate head of *A. tamari*, showing a less compact structure than *b.* *f.* Globose head of *A. Wentii*, with heavy-walled stalk.

P is a more slender, delicate form than C, with crowded stalks, and also loses its color quickly.

A. oryzae var. *basidiferens* Costantin and Lucet[16] differs from *A. oryzae* as described by Wehmer in having both primary and secondary sterigmata. Since the authors of this variety had no other cultural experience with *A. oryzae*, and since all cultures we have seen appear to show this character, it seems best to us to introduce this observation as an emendation to the description of *A. oryzae* instead of recognizing the validity of a variety.

A. pseudoflavus Saito[17] appears to represent some one of the races intermediate in length of stalk between *A. flavus* and *A. oryzae* but having the color, usually simple sterigmata, and size of conidia found in *A. oryzae*. *Aspergillus micro-virido-citrinus* Costantin and Lucet[18] differs from *A. pseudoflavus* only in its smaller conidia. *A. gymnosardae* Yukawa[19] was found upon the fermented fish product, katsuobushi, in Japan. The description clearly marks it as also intermediate in structure between *A. flavus* and *A. oryzae*. We have not been able to identify either of these forms with certainty, although we have had several strains in culture which occupy such an intermediate position.

Similarly, another strain appears in our collection once from America

---

[16] *Loc. cit.*, p. 167.

[17] Saito, K. Centralbl. Bakt. II, 18: 34. figs. 15–18. 1907.

[18] *Loc. cit.*, p. 158.

[19] Yukawa, M. Jour. Coll. Agr. Tokyo 1: 362. Tab. XVIII, figs. 1–7. 1911.

(no. 129), and once contributed by Hanzawa from Japan. This form in culture is *citron green* to *lime green* (Ridgway XXXI. 25''). It has short, crowded stalks like *A. fumigatus* with small heads, with mostly a single series of sterigmata and conidia predominantly small, 3 to 4 μ, few reaching 5 to 7 μ in diameter.

A. parasiticus Speare. Speare[20], working in Honolulu, found one of these forms parasitic upon the mealy bug of sugar cane (*Pseudococcus calceolariae* Mask), and described his strain as *A. parasiticus*. A culture with the same characters was isolated by one of us from mealy bugs obtained from Demerara; another culture was isolated from cane sugar in New Orleans by Kopeloff. However, other strains of the *flavus* group were isolated by Johnston[21] from mealy bugs in Porto Rico; reinfection experiments by Johnston, while not conclusive, established a presumption of infectiousness as a strain or race character unconnected with the specific morphology of Speare's *A. parasiticus*.

Speare's organism when grown on Czapek's solution agar differs from the commoner forms of *A. flavus* in its predominantly greener color (near *ivy green* Ridgway XXXI. 25'' m), in short stalks usually 200 to 400 μ long, in heads with usually a single series of sterigmata 7 to 10 μ long by 3 to 5 μ. No sclerotia have been seen. The mycelium is uncolored. Otherwise the characters are those of the *A. flavus* group.

A. effusus Tiraboschi.[22] Cultures of a cottony, floccose type have been obtained from widely separated sources (no. 130 from Dr. B. F. Lutman, Burlington, Vermont; no. Sc. 171 in corn meal from Indiana; no. 2750 isolated by Johnston from mealy bugs in Porto Rico). Superficially these cultures show little relation to *A. flavus*. Microscopic examination of heads and spores, however, shows close relationships.

Characterization of A. effusus: Colonies on Czapek's solution agar with sucrose, broadly spreading, effused floccose, or cottony, white becoming slowly dirty yellowish or in restricted areas greenish yellow; reverse and agar yellowish. Stalks either *A. flavus*-like arising directly from the substratum, up to 500 μ long and frequently with large radiate heads, or predominantly in the form of branching, trailing, thick-walled hyphae, each segment consisting of a long, thick-walled, fertile cell bearing a perpendicular branch (stalk) usually less than 100 μ long by 5 to 10 μ in diameter, with walls pitted and sometimes granuliferous, bearing usually columnar heads; vesicles in small heads up to 20 μ in diameter, occasionally larger, sterigmata in one series, 6 to 10 μ by 3 to 5 μ, mostly on apex only of vesicle; larger heads with either simple or branched sterigmata as in *A. flavus*. Conidia pitted as in *A. flavus*, pale yellow, rather thin-walled, pyriform to globose, varying in size in the same culture from 3 by 4 μ to 5 by 7 μ. Neither sclerotia nor perithecia were found. Colonies grew well in all common media, grew better at 37° C. than at 20° C., liquefied plain gelatine (gelatine in distilled water) with yellow color in the liquid.

---

[20] Speare, A. T. Fungi parasitic upon insects injurious to sugar cane. Hawaiian Sugar Planters Exp. Sta. Path. and Phys. Ser. Bull. **12**: 30. 1912.

[21] *Loc. cit.*, p. 15.

[22] Tiraboschi, C. Atti Terzo Congresso Pellagrologico Italiano, p. 142. Milano, 1906; diagnosis in Annali di Botanica **7**: 16. 1908.

The puzzling appearance of these cultures led to studies in morphology and to extensive experiments scattered over nearly ten years. Comparative study of all species obtainable shows that the stalk throughout the genus Aspergillus originates as a mycelial cell transformed into a spore-producing organ. The cell enlarges in diameter and its wall becomes thickened. The stalk arises as a branch approximately perpendicular to the course of the original cell which remains in the hypha as a kind of foot. Usually the stalk, beginning with the diameter of its foot cell, broadens upward and lengthens, hence becomes many times the size of its foot. In *A. effusus* the foot cell is frequently very long, branching and connected with other foot cells to form a trailing, fertile hypha from which the stalks arise as short branches. Selective transfers from Lutman's strain (no. 130) showed the possibility of separating a race which appeared to be the usual form of *A. flavus* and another in which the heads were borne only on the trailing type of fertile hyphae among cottony white masses of sterile hyphae. The hypotheses of symbiosis and of parasitism were both tested through many transfers without result. The heads on all series of cultures maintained the essential morphology of *A. flavus*, although the colony characters diverged widely. More recently, a transfer was made from a stock culture several months old which was grown upon Czapek's solution with the addition of 5 percent sodium chloride, 10 percent sucrose, and 3 percent agar. The original strain (Lee 108) had maintained the cultural appearance of *A. flavus* as received from Wehmer, through successive transfers for about four years. This transfer produced a floccose type of colony with some *A. flavus* type of fruiting at the edges. Transfer from the whitest areas in this culture produced the typical white colony described above; transfer from an area showing few small heads produced a mixed colony; transfer from selected heads appearing to be *A. flavus* gave a pure *A. flavus* colony. All of the experimental work supports the hypothesis that the floccose types represent mutants from the typical *A. flavus*, which were probably induced in the last experiment in similar manner to the mutations of *A. niger* as described by Schiemann.[23]

The description given by Tiraboschi (*loc. cit.*) for *A. effusus* probably applies to these cultures. *A. effusus* was isolated by Tiraboschi from spoiled corn products.

Nomenclature. Aspergillus flavus was first described by Link[24] in terms vague enough to baffle any attempt at certain identification. The habitat given was herbarium specimens. Our own search over many lots of moldy plants in herbaria, together with interpretation of the name *flavus,* suggested that some one of the *Aspergillus herbariorum-repens-Amstelodami* series in the sense of Mangin[25] was the basis of Link's description. Specimens have been actually found in several series of exsiccatii labeled *A. flavus* but clearly consisting wholly of *A. repens.* However, Link records his acquaintance with the green Aspergillus of the herbarium under the name *A. glaucus.* The universally distributed yellow perithecial forms which were later connected with the common green forms by De Bary were known to Link under the name Eurotium. Clearly, then, Link believed that he had some organism which he found commonly upon badly dried herbarium specimens and which was yellow enough in contrast to *A. glaucus* to justify the name *A. flavus.* The conidial forms of the *A. glaucus* group do not show a yellow color factor. Following De Bary,[26] there is, moreover, the widespread use of this specific name *A. flavus* Link for our series of yellow-green forms which are universally distributed.

---

[23] Schiemann, E. Mutationen bei *Aspergillus niger* van Tieghem. Zeitschr. Indukt. Abstam.- u. Vererbungslehre 8: 1–35. 1912.

[24] Link, H. F. Observations in: Ordines plantarum naturales. Gesellschaft Naturforschender Freunde zu Berlin, Magazin 3, p. 16. 1809. This is usually cited Link. Obs. p. 16. 1809. The description in full follows: "Caespitibus laxis, floccis albis erectis, capitulis junioribus albis, adultioribus flavis. Frequens in plantis siccis herbariorum."

Wilhelm,[27] Schroeter,[28] and Wehmer[29] base their use of this name upon Brefeld's specimens distributed as no. 2135 in Rabenhorst's[30] Fungi Europaei. A comparison of Brefeld's specimens with a culture obtained in Wehmer's laboratory in 1905 and still maintained by us in culture shows them to be morphologically identical. This strain agrees with the characterization of *A. flavus* in Wehmer's Monograph. Costantin and Lucet[31] reach the same general conclusion without record of having seen the specimens, and add the comment that this identification constitutes the perpetuation of a tradition that this particular strain is *A. flavus* Link. This identification is promptly discarded by them and its name changed to *A. Wehmeri* Cost. et Lucet.[32]

With the study of the culture from Wehmer as a basis, the distribution of this and closely related strains has been followed for about ten years. Numerous cultures have been isolated and compared from diverse sources. Molds with essentially this morphology have been sent to us in series of soil cultures made by Esten and Mason in Connecticut, by Miss Dale in England, by Johnston in Porto Rico, by Waksman in New Jersey, by McBeth and Scales in Washington, by Werkenthin in Texas, and by Hartley from coniferous seed beds in Kansas. They have been isolated by us many times from miscellaneous foodstuffs, especially the cereals both as whole grain and as milled products, and more recently have been found abundantly in the soy-bean fermentation products of China and Japan.

---

[25] Mangin, L. Ann. Sci. Nat. Bot. IX, **10**: 303–371. 1909.

[26] DeBary, A. Beiträge zur Morphologie der Pilze. III^{te} Reihe, 2te Abt., p. 20. 1865.

[27] Von Wilhelm, K. A. Beiträge zur Kenntniss der Pilzgattung Aspergillus. Inaug. Diss. Strassburg. Berlin, 1877.

[28] Schroeter, J. Cohn's Kryptogamenflora von Schlesien 3^2: 216. 1893.

[29] *Loc. cit.*, p. 81.

[30] Rabenhorst. Fungi Europaei Edit. Nov. ser. II. 1875.

[31] *Loc. cit.*, p. 152.

[32] *Loc. cit.*, p. 169.

Members of this series have been reported in the study of infections in the human ear. Certain of these forms have produced lesions and death when injected into experimental animals. Costantin and Lucet[33] review the literature of such pathogenicity and offer a key to species based upon their review of injection experiments with rabbits and fowls. The structural characters cited by them represent fairly well the range of variation within the group. Sclerotium formation is used to separate *A. flavus*, attributed by them to Wilhelm, from the other forms. In our experience sclerotium formation is not limited to any morphological section of the group. Moreover, *A. Wehmeri* of Costantin and Lucet is a manuscript species based upon *A. flavus* of Wehmer's monograph. It was not studied by them in culture. Both Wilhelm and Wehmer based their use of the name upon the usage of Brefeld as determined by examination of the same cultural material distributed by Rabenhorst.[34] We have seen this material, and it corresponds satisfactorily with the characters given by Wilhelm and Wehmer. If the name *A. flavus* is to be held in the sense of Wilhelm, *A Wehmeri* is clearly a synonym.

A. flavescens of Wreden[35] was not cultivated. The size of the spores and the coloration of the stalk reported caused us to believe that it belonged elsewhere.[36] Wehmer[37] cites Lichtheim as having compared *A. flavescens* with *A. flavus* Link as understood by Brefeld and having found them identical. Lichtheim, however, uses the name as interpreted by Eidam, which is probably but not necessarily identical with the usage of Wreden. Certain organisms from ulcerated ears clearly belonged to this series. The morphology given by Costantin and Lucet for *A. Siebenmanni* and *A. micro-virido-citrinus* is not uncommon in cultures from the group except as to color. It will be shown later that the green factor in colony color is suppressed when fermentable carbohydrates are omitted from the substratum.

The range of morphology cited by Costantin and Lucet was assumed by them to establish a presumption of pathogenicity to warm-blooded animals for the whole group. The infection experiments reported from different sources were intravenous with positive lesions. It is noteworthy that they found their *A. oryzae* var. *basidiferens*[38] to be pathogenic also to the rabbit by the same kind of inoculation. This is consistent with a common morphology in *A. flavus* and *A. oryzae* as considered in this paper. Double sterigmata, used by Costantin and Lucet as varietal characters, are not the exception but the rule in the saké organism in which only occasional cultures show only simple sterigmata. In the rice and soy fermentation industries of Japan the workman's eyes, ears, nose, throat, and skin abrasions are constantly exposed to Aspergillus spores. Dr. Takahashi and Dr. Kita (personal communications), however, report absolutely no infections. Our cultures show a wide range of varieties of the *Aspergillus flavus* series to be present. Intravenous injection, doubtless, has value in demonstrating the possible activity of an organism when so inoculated, or perhaps in lesions already established by other agencies, without proving active pathogenicity.

---

[33] *Loc. cit.*, pp. 151–163.

[34] *Loc. cit.*, no. 2135. 1876.

[35] Compt. Rend. Acad. Sci. Paris **65**: 368. 1867.

[36] Thom, C., and Church, M. B. Amer. Jour. Bot. **5**: 100. 1918.

[37] Wehmer, C. Centralbl. Bakt. II, **2**: 148. 1896.

[38] *Loc. cit.*, p. 167.

Wreden and Siebenmann use the name *A. flavescens* for organisms found in the human ear. Wreden's description lacks essentials for identification perhaps, but Lichtheim certainly had an organism of this group from infected ears. Siebenmann, using the same name, gives details which definitely ally his form with either *A. flavus* or *A. tamari* (see discussion of *A. tamari* later). Wilhelm clearly has a sclerotium-producing strain closely allied to the material studied by Brefeld. Wehmer, whose organism we have in culture, had a different but closely related strain which rarely if ever produces sclerotia. Costantin and Lucet[39] appear also to have had but one strain of the same series, which they described as *A. micro-virido-citrinus*. Cultural observations limited to single strains in a group varying as widely as this may easily lead workers unacquainted with other material to believe they have distinct species. When comparison of hundreds of cultures from separate sources has bridged the gap between these forms, it is doubtful if any effort to maintain such species is desirable.

There appears to be no valid reason for rejecting the name *A. flavus* for the cosmopolitan organism studied by Brefeld and Wehmer and as tentatively covering many strains with minor variations from such a type. *A. oryzae*, *A. parasiticus*, and *A. effusus* are morphologically recognizable varieties or species which are certainly closely related to the cosmopolitan group of which the organism described by Brefeld and Wehmer and believed by them to be *A. flavus* Link may be called the type.

In reaching this conclusion, many series of cultures were made with a large number of strains selected to represent the widest range of variation found in our collection. These cultures included an extensive variety of culture media; the bark of Castanea, Liriodendron, Platanus, and Tsuga, oatmeal agar with and without sugar, potato plugs, beef extract peptone agar, egg albumen, beef plugs, loam, rice, cooked soy beans mixed with ground and roasted wheat, Czapek's solution with cerealose instead of sucrose. The following paragraphs describe some points observed which seem to be worthy of note.

Czapek with 50 percent saccharose: Twenty-seven strains of *Aspergillus flavus* grown on Czapek solution agar containing 50 percent saccharose grew for all practical purposes the same as if on unmodified Czapek solution agar. In 3 strains of *A. effusus*, fruiting was increased and the conidial areas and the reverse were *citrine* in color. The two strains of *A. oryzae* were more brownish in color and the conidiophores were short as compared with the growth of the same strains on the standard Czapek solution agar. *A. terricola* var. *Americana* grew sparsely on this medium.

---

[39] *Loc. cit.*, p. 158.

Fish agar (halibut): Nine strains of *A. flavus* when grown on fish agar for two months developed only a few brown heads; nine other strains of the same species developed only white mycelium. *A. oryzae* and *A. effusus* also did not fruit. *A. tamari* and *A. Wentii*, however, showed fruiting at first *old gold* in color and scarcely spreading beyond the mark of the streak.

Beef plugs: Plugs of fresh beef were cut with a cork borer and placed in tubes ordinarily used for potato plugs. The sterilization was fractional. At the end of two weeks six of the more common strains which develop conidial areas near in color to *Krönberg's green* on Czapek solution agar were *olive ocher* (Ridgway XXX. 21″) on the beef, four others ran through *olive ocher* to *old gold* (Ridgway XVI. 19′ i); three strains changed from *olive ocher* to other tints and shades of orange and yellow; one strain never developed any deeper color than *deep colonial buff* (Ridgway, XXX. 21″ b). All the green color was, therefore, eliminated from these strains of *A. flavus* when grown on cooked beef, with the exception of one strain which became *lime green* after it had appeared *olive ocher*. The early growth of *A. parasiticus* was at first *mignonette green* and later *olive lake* (Ridgway XVI. 2′ i), a shade with no green; a yellow green strain (no. 129), possibly *A. microvirido-citrinus*, corresponding with *A. terricola* of the brown series, was *colonial buff* (Ridgway XXX. 21″); and strains of *A. effusus* at the end of two weeks were *chamois* (Ridgway XXX. 19′ b). No green color was exhibited in the whole group.

Plain agar (bacteriological): The *A. flavus* group when planted on plain agar produced color practically as when grown on beef plugs. The green factor was not suppressed as completely, however. It was more evident during the first few days of growth, and seemed to disappear except in the same instances noted under beef plugs.

Synthetic agar (Currie's):[40] Thirteen of the *A. flavus* strains and *A. parasiticus* developed the green color more intensely with a reduction of yellow, when grown on this agar. They developed such shades and tints as *Kildare green* (Ridgway XXI. 29″ b), *Rainette's green* (Ridgway XXI. 27″ i), *cress green* (Ridgway XXI, 29″ k), etc. Six similar strains grew as if on Czapek solution agar, as did also *A. effusus*.

In these experiments the colors reported range from mixtures of yellow and orange to various combinations of yellow and green. The reversible factor appears to be green. Kita[41] reports similar observations. In describing *A. pseudoflavus*, Saito[42] observed that exposure to ammonia would destroy the green color entirely, leaving yellow or yellowish brown. Subsequent exposure to vapor of acetic acid restored the green color to this colony. The test used by Saito was applied to *A. flavus* (no. 108, the Wehmer strain), *A. oryzae* (the saké organism), and *A. parasiticus*, which represent the widest range of differences in our collection. Each of these strains gave the reactions described by Saito for *A. pseudoflavus*. The test was repeated with hydrochloric acid substituted for acetic acid. The correlation of the green color with the acid was clearly brought out. These strains grown in Czapek's solution agar are, respectively, *A. oryzae* about *lime green*, *A. flavus* near *Krönberg's green*, and *A. parasiticus* close to *ivy green*, all in column 25″, Plate XXXI of Ridgway's tables. When exposed to the fumes of hydrochloric acid the green color was intensified, reaching colors given in column 29″ of the same plate with the deepest areas reaching the same intensity, *cress green*. When the same cultures were exposed to ammonia, all of the green disappeared, and the colors remaining corresponded with combinations in column 21″, Plate XXX, varying from *deep colonial buff* to *olive* in the deepest areas.

---

[40] $(NH_4)H_2PO_4$, 2.0 gms.; KCl, 0.2 gm.; $MgSO_4$, 0.1 gm.; cane sugar, 30 gms.; agar, 15 gms.; H_2O, 1 l. Formula used by Dr. J. N. Currie.

[41] Kita, Gen-itsu. Ueber die Konidienbildungsfaehigkeit einiger Varietaten des *Aspergillus Oryzae*. Original Communications, 8th International Congress of Applied Chemistry 14: 95.

In the experiments previously described the green colors are found present in marked degree only upon media containing sucrose or some other fermentable carbohydrate. In cultures upon beef, fish, egg, and soy beans, which lack carbohydrates or are very poor in fermentable carbohydrates, the greens were absent or nearly so throughout the series. In the mixtures of fermentable carbohydrates and proteins there are evidently simultaneous acid and alkaline fermentations which tend to neutralize each other as described by Ayers and Rupp.[43]

The early development of conidia always shows some development of green in such cultures. When litmus is used in the culture medium, these early stages are always accompanied by the acid or red reaction. Many such cultures eventually lose all their green color, but this loss is always preceded by change in reaction. If a series of these strains grown upon a single medium show different shades of green, these shades of green are thus indications of the relative acidities reached by the culture. Some correlation between the typical color shown by a colony and the progressive changes in the reactions of the substratum, and possibly even of cytoplasm, is indicated.

The substratum influences the development of such saprophytic fungi in numerous directions. The gross appearance of a pure mold culture may be entirely altered through the influence of the medium on which the fungus is growing. The dimensions of certain structures in an Aspergillus may be altered by a change in the medium, while those of other structures may remain constant as long as the medium is not totally inadequate or does not contain deleterious substances. Aborted or unrecognizable types of structure result from conditions positively inhibitive for normal growth. Conidial growth, sclerotia, or perithecia, each may be totally or in part suppressed or their production may be stimulated by the nutrient provided. The structure and markings of the stalk wall, general shape, markings, and range of size in conidia are fairly stable within the strain or species and fall within certain limits which for practical purposes do not vary. The length of the stalk, diameter of the vesicle, the dimensions of the primary sterigmata, and, within limits, the spore measurements are influenced by the substratum. Wall markings cannot be said to vary with the nutrient supplied, although their conspicuousness varies slightly from culture to culture doubtless through the pressure of several factors. The alterations in these latter structures are never permanent. They are dependent entirely on the substratum. Certain media stimulate in such fashion as to cause an increase in dimensions, others a dwarfing. The majority of culture media cause each strain to develop to a size falling within fairly well-defined limits.

---

[42] Saito, K. Microbiologische Studien über die Zubereitung des Batatenbranntweines auf der Insel Hachijo (Japan). Centralbl. Bakt. II, **18**: 30–37. figs. 1–22. 1907.

[43] Ayers, S. H., and Rupp, P. Simultaneous acid and alkaline fermentations rom dextrose and the salts of organic acids respectively. Jour. Infec. Dis. **23**: 188–216. 1918.

Aspergillus Wentii and Related Forms

Aspergillus Wentii was described by Wehmer[44] and has been widely distributed in culture from the Centralstelle at Amsterdam. Extensive cultural studies show the species to differ from the characterization given by Wehmer in the quite general presence of both primary and secondary sterigmata. Identity of the Amsterdam strain with Wehmer's material is hardly questionable. Cultures with the same morphology have been found by us upon moldy corn grains, upon moldy cotton cake from Georgia, (4230) within a temechee nut from Brazil, upon cubebs from Singapore, and received (4204.16c) from China through the kindness of Mr. Chung, from Hanzawa (4291.32) in Sapporo, (4186.34) from Panama collected by Dr. Thaxter, from Oregon soil (4078.0–5) collected by Waksman. One culture was received from Ohio Experiment Station, one from Prof. R. A. Harper. Although these forms differ in details of reaction and appearance, the morphological characters found mark them as a natural group.

Characterization of A. Wentii Wehmer. Colonies on Czapek's solution agar with cane sugar, deeply floccose, spreading, with sterile hyphae white or yellowish, and with heads white at first, changing through *cream, cream buff, honey yellow, old gold*, to *light brownish olive, medal bronze*, or in old cultures sometimes *snuff brown* (Ridgway, column 19, Plates IV, XVI, XXX, and Plate XXIX 15″ K; recorded as *coffee-brown* to *chocolate brown* by Wehmer), and in some strains producing large masses of aerial mycelium which in tubes may fill the lumen 3 cm. above the substratum; reverse of colony yellowish at first, becoming reddish brown when old; agar frequently colored yellow; stalks 2 to 3 mm. or up to 5 mm. long, commonly 10 to 12 μ or sometimes up to 25 μ in diameter, inconspicuously 1- to 2-septate, with walls colorless, up to 4 μ in thickness, and smooth, often studded with droplets in young cultures, enlarged at tips to vesicles widely varying up to 80 μ in diameter; heads large, yellow to brown, stellate (or globose fig. 1, f); sterigmata usually in two series, primary varying greatly, 6 to 8, occasionally to 15 μ by 3 to 5 μ, in extreme cases up to 60 μ by 8 to 10 μ; secondary 6 to 8 by 3 μ (a single series is recorded by Wehmer 15 by 4 μ). Conidia pyriform to globose, usually about 4 by 5 μ, less commonly up to 5 to 5.5 μ by 5 to 6 μ (4.2 to 5.6 μ, Wehmer), with walls thickened to leave pits or furrows on the surface arranged roughly lengthwise of the spore chain, frequently appearing smooth or nearly so with low magnifications, commonly more or less plasmolyzed when treated with 95 percent alcohol.

---

[44] Wehmer, C. Eine neue technische Pilzart Javas. Centralbl. Bakt. II, 1: 150. 1895.

Perithecia not found. Sclerotia limited to more or less undefined masses of thick-walled cells occurring occasionally, not uniformly. Cultural optimum below 37° C. in all strains tested. Gelatin liquefied in cultures, both with and without sugar.

The Java culture originally sent by Went to Wehmer was used in rice and soy fermentation on that island by Chinese workmen. The strains since found resemble the Amsterdam strain in their range of color changes, smooth, thick-walled stalks without pits, stellate heads, double sterigmata, and in the *A. flavus*-like marking of the conidial wall. The mass of sterile mycelium above the colony in typical test-tube cultures of the organism, as described by Wehmer, is present in the Amsterdam strain, but lacking or only partially or occasionally present in some of the strains. This overgrowth of mycelial masses with fruiting at several levels through a considerable period becomes more prominent upon potato plugs. A gradation from the type strain of Wehmer to those lacking this character in Czapek solution agar cultures, combined with the common structural characters cited, justifies the extension of our idea of *A. Wentii* to include these forms at least as varieties of a widespread natural group.

Inui[45] described *A. perniciosus* as found in awamori-koji without giving details of stalk and spore markings but comparing the stalks with those of *A. Wentii* and *A. luchuensis* both of which we have in culture. *A. perniciosus* belongs probably in the group with *A. flavus* and *A. oryzae* on account of the data given as to color and habit, which correspond to certain of the variant strains contributed by Dr. Takahashi.

A. TAMARI AND ALLIES

A second brown series of forms is more closely associated in occurrence and in habit with *A. flavus* and its allies than is *A. Wentii*. Many cultures in this series have been obtained in forage and feeding stuffs, from the Oriental soy fermentations, upon food, in soil, and growing as laboratory contaminations. Cultures have been thus obtained from China, Japan, and South America as well as from many points in the United States. These brown forms are characterized by absence of true green in color, by stalks prominently pitted especially toward the apex, and by conidia tuberculate at the distal end in the chain, rough, showing on detailed examination firm, fairly thick, and not pitted inner walls, thin, vesicular outer walls fitting rather loosely over masses of branching, more or less irregularly-arranged bars of yellow-brown substance. In size of colony, habit, and appearance aside from color, these forms resemble *A. flavus*. In the markings of conidia they suggest *A. niger*.

---

[45] Inui, T. Jour. Coll. Sci. Imp. Univ. Tokyo **15**: 473. 1901.

Characterization of A. tamari Kita (from our culture no. 4235. I–2). Colonies on Czapek's solution agar with cane sugar spreading broadly, with vegetative hyphae mostly submerged, with fruiting areas at first colorless, then passing through orange-yellow shades to brown in old colonies (variously *Isabella color, light brownish olive, buffy citrine, medal bronze,* or *raw umber.* Ridgway *loc. cit.*, column 19, Plates XXX, XVI, IV, and column 17, Plate III), not showing true green; reverse uncolored or occasionally pinkish; stalks arising from submerged hyphae, up to 1 to 2 mm. in length, becoming several millimeters in length upon corn or other concentrated media, 10 to 20 μ in diameter, increasing in diameter toward the apex and passing rather abruptly into vesicles, with walls rather thick, 1 to 2 μ, becoming abruptly thinner at the base of the vesicle, pitted more prominently in upper than lower half (often appearing as rough or echinulate with low magnifications) and frequently showing irregular thickenings within; vesicles 25 to 50 μ in diameter with fairly thin walls which frequently crush in mounts; heads varying greatly in size in the same fruiting area, from more or less columnar to nearly but not completely globose (fig. 1, *a*, *b*, and *e*), and up to 350 μ in diameter, with radiating chains and columns of conidia; sterigmata, one series in small heads, two series in large heads, primary commonly 7 to 10 by 3 to 4 μ, becoming 20 to 35 μ long in gigantic heads upon corn; secondary 7 to 10 by 3 μ; conidia more or less pyriform toward globose, tuberculate especially at the distal end in the chain, 5, 6, occasionally up to 8 μ in diameter, rough from prominent masses and bars of orange-yellow coloring matter deposited under the loose outer wall upon the firm inner wall. Sclerotia occasionally produced.

These forms are widely distributed and resemble *A. Wentii* in color but have the gross morphology and habits of *A. flavus*. Their conidia show color bars resembling those of *A. niger* in formation and in solubility in water.

In examining the exsiccati, collections have been repeatedly found upon corn grains (*Zea Mays*) with the color and conidial markings of *A. tamari*, but with long stalks whose thick walls obscured the pitting except close to the head and with primary sterigmata 20 to 30 by 5 to 6 μ and larger conidia. Cultures from another strain (S_3 from sardine paste) when grown upon Czapek's solution agar showed vesicles about 35 μ in diameter, primary sterigmata 8 to 14 by 3 to 5 μ, and conidia 5 to 6 μ in long axis. When grown in unsterilized, clean corn for two weeks, this organism showed vesicles 100 μ in diameter, primary sterigmata 25 to 35 by 5 to 9 μ, and conidia 8 to 9 μ in long axis. The secondary sterigmata seem to vary much less under such differences of environment than the primary sterigmata. Transfer from the corn back to Czapek's solution agar gave the original measurements. Such cultural experiences emphasize the necessity of using a standard medium as the basis of comparative studies of saprophytic organisms and destroy all faith in the significance of measurements to the fraction of the micron from miscellaneous cultures. At best a range of measurements must be allowed for in the most carefully standardized culture work.

Cultures with this group morphology vary in shades of color enough to separate different strains when observed in parallel culture. A ten-day petri-dish culture (no. 3565) showing its outer zone *maize yellow*, intermediate zone *orange-citrine*, and deepest area *medal-bronze* (Ridgway *loc. cit.*, IV. 19 f, k, and m) was inverted over a dish of hydrochloric acid. The shade of mixed yellow and orange quickly changed to *Saccardo's olive* (Ridgway III, 19 m). The petri-dish culture was then placed over a dish of strong aqua ammonia and quickly changed toward a shade between *raw umber* and *Brussels brown* on the same plate (Ridgway III). This color approximates that of very old cultures of members of this series which, in common with those of *A. flavus*, become more alkaline with age. The response in *A. tamari* is not as conspicuous as the change in green shades of the *A. flavus* group, but clearly indicates that differences in color of the same culture at different ages and between different strains of the series is due to variation in the reactions induced by the metabolic activity of the organism. These differences vary with the composition of the medium and with the characters of the race or strain studied, but clearly indicate close relationship among the forms.

A. citrisporus von Höhnel. Another form having characters of this group was sent by Dr. Thaxter[46] from excrement of caterpillars. In Czapek's solution agar cultures, this form showed colorless submerged mycelium, and conidial areas at first yellow, then golden, and finally fulvus; stalks 1 to 2 mm. high, up to 20 to 25 μ in diameter, turgid when young, often collapsing in age or when exposed to dry air, septate, with walls thin (1 μ or less mostly), appearing to be studded with fine granules when examined directly (in air) but appearing smooth in liquid except when high magnification and great care are used to demonstrate the abundant pitting; with heads up to 500 μ in diameter; vesicles 30 to 50 μ in diameter, nearly globose, and fertile over nearly the entire surface; sterigmata in one series, 8 to 12 μ by 3 to 4 μ with long, loosely radiating conidial chains. Conidia yellow or golden, then brown, lemon-shaped, 5 to 9 by 5 to 6 μ, rough from irregularly branching ridges of yellow to brown coloring matter between the inner and outer wall.

Sclerotia are occasionally found.

Cultures from excrement of caterpillars by Dr. Roland Thaxter.

---

[46] Culture received bore the manuscript name *A. chrysospermum*.

A. terricola Marchal. A culture belonging to this group was isolated by Scales[47] from redland soil in Georgia and discussed under the name *A. terricola* Marchal.[48] This culture shows the characteristic morphology of the group but differs in shade of color and in it's smaller measurements of stalk, vesicle, and head. It bears about the same relation to *A. tamari* as described above that *A. parasiticus* of Speare does to *A. flavus* as accepted by Wehmer. The color recorded by Marchal, *umbrinus*, readily separates the culture when compared to the colors found in the other members of the group, but the composition of this color shows close relationship to that of *A. tamari* when analyzed in Ridgway's plates.

Scales' culture was submitted to Marchal, who designates the form as *A. terricola* var. *Americana* Marchal, distinguished as follows:

A. terricola var. **Americana** Marchal n. var. "The dimensions of the vesicles 14 to 20 instead of 30 to 50, of the sterigmata 5.6 to 10.5 by 2.2 μ instead of 12 to 15 by 4 to 7 μ; the spores only very delicately verrucose, separate your fungus from *A. terricola*."

A culture nearly related in color and morphology was described by Mrs. Patterson[49] as *A. umbrinus.* The original material and cultures appear to have been lost, and the description lacks details which would decide its exact status.

Nomenclature of the A. tamari series. Kita[50] described as *A. tamari* a culture discovered as a contamination in a Japanese fermented product, tamari-koji. Numerous cultures of American origin show the morphology of *A. tamari* Kita. This identification has been confirmed by conference in which Kita examined a whole series of these strains in culture. The possibility that the organism had been previously described remained for consideration. From its brown color, its identification with *S. castanea* Patterson[51] seemed possible until Mrs. Patterson's exsiccati had been examined and were shown to belong to the *A. niger* group. Upon some media the young heads become definitely orange before becoming brown. This change, together with the pitted stalk and double sterigmata, suggested *A. fulvus* Montagne[52] which was described in connection with silkworm diseases in southern France in 1849, but has not been reported since. Von Höhnel[53], however, described *A. citrisporus* with similar heads from excrement of caterpillars. The conidia in this form are at first golden yellow, then fulvous, and lemon-shaped instead of globose as given for *A. fulvus*. Dr. Thaxter's culture already described agrees, therefore, closely with *A. citrisporus* as described by von Höhnel. A specimen in the herbarium of the New York Botanical Garden collected by Peck upon excrement of caterpillars at Sandlake, N. Y., appears to be identical with Dr. Thaxter's culture. The characters reported in common for these four strains from caterpillars suggest either identity or the existence of a series of related forms widely distributed and associated with caterpillars. The strain in culture is distinguishable from the forms included under *A. tamari*, although evidently related to it.

---

[47] Scales, F. M. The enzyms of *Aspergillus terricola.* Jour. Biol. Chem. **19**: 259–272. 1914.

[48] Marchal, É. Sur une espèce nouvelle du genre Aspergillus Micheli, *A. terricola.* Rev. Mycol. **15**: 101–3. 1893.

[49] Patterson, F. W. New species of fungi. Bull. Torrey Bot. Club **27**: 284. 1900.

[50] Kita, G. Einige japanische Schimmelpilze. Centralbl. Bakt. II, **37**: 433–452. 1913.

[51] Patterson *loc. cit.*; also exsiccati in pathological collections, U. S. Dept. Agr.

[52] Montagne, C. Plantes cellulaires: Cent. VI, no. 82. Ann. Sci. Nat. Bot. III, **12**: 298. 1849.

[53] Von Höhnel, F. Fragments zur Mykologie, I. Mittheilung. Sitzungsber. K. Akad. Wiss. Wien, Math.-Naturw. Kl. I, **111**: 987–1056. 1902.

The name *A. tamari* Kita is retained here for the group common in food-stuffs and fermentation products, *A. terricola* var. *Americana* Marchal for the soil organism isolated by Scales, and *A. citrisporus* von Höhnel for the caterpillar organism. These are readily separable in culture.

The following paragraphs give specific relations of species and strains of the Aspergilli here under consideration to various culture media.

A. tamari on seed corn. A reversible variation due to corn as a substratum was noticed with strains of *Aspergillus tamari*. We first noticed abnormally large sterigmata and occasionally conidia of this species in exsiccati. For example, a fungus of this type growing on grains of "*Zea Mays*, Waco, Texas, 2–26–1909, Comm. F. Hedges, Pl. Disease Survey," and deposited among the exsiccati of the Department of Agriculture, showed sterigmata with primaries 20 to 30 by 5 to 6 μ and wedge-shaped, secondaries 8 to 9 by 3 to 3.5 μ. S_3 Asp (a strain recovered from canned sardine paste) had on a Czapek agar slant a vesicle measuring 35 μ, primary sterigmata 8 to 14 by 3 to 5 μ, secondaries normal, conidia 5 to 6 μ. Material from this tube was inoculated into a bottle of unsterilized, clean corn. Two weeks later the new culture showed vesicles 100 μ, primary sterigmata 25 to 35 by 5 to 9 μ, secondaries 15 by 3 to 4 μ, conidia 8 to 9 μ. The same observations were made with two other strains of this species. Retransfer from the corn culture to Czapek solution agar produced what is considered typical growth using Czapek as a standard.

Tests for phenolic substances with an aqueous solution of ferric chloride align the *Aspergillus tamari* group closely with the *A. flavus-oryzae* group as to this particular chemical reaction. All strains of these three species which were tested showed a red reaction, varying from brownish red to a rich wine-red. This variation is dependent both on the ability of the individual strain to produce a given quantity of a phenolic substance and on the ingredients of the culture medium. *Aspergillus Wentii* gives, however, a clear yellow color when tested with ferric chloride. The best results were obtained by using a transparent liquid medium[54] from which a sample of from one to several cubic centimeters could be withdrawn with a sterile pipette. These experiments are merely a preliminary step in an investigation of the production of phenols by molds. Dr. J. F. Brewster of the Laboratory of Biological Investigations is at present engaged on this research.

---

[54] (1) Ordinary household rice extracted in water at 58–60° C. for one hour. (2) Tap water, 1 liter; starch (soluble), 1 per cent; ammonium nitrate, 0.05 percent; K_2HPO_4, 0.05 percent.

Key to species described from culture.

- I. Conidia pitted-areolate (determinable only by oil immersion):
  - *A.* Colonies typically yellow-green at first, shading to yellowish brown in age. *A. flavus-oryzae* series:
    - Aerial growth of fertile hyphae only:
      - Stalks long, 2 to several mm.: *A. oryzae.*
      - Stalks 400 to 600 μ, rarely 1,000 μ: *A. flavus.*
      - Stalks 200 to 500 μ, crowded: *A. parasiticus.*
    - Aerial growth, both vegetative hyphae and fertile hyphae: *A. effusus.*
  - *B.* Colonies yellow to brown, never green: *A. Wentii.*
- II. Conidia bearing brown color-bars:
  - Stalks conspicuously pitted, thick-walled:
    - Stalks 500 to 1,000 μ long: *A. tamari.*
    - Stalks 300 to 600 μ long: *A. terricola.*
  - Stalks obscurely pitted, collapsing in age: *A. citrisporus.*

Suggested relationship of species elsewhere described from culture but not identified by us:

- *A.* Belonging to *A. flavus-oryzae* series:
  - 1. Gigantic and floccose types related to *A. oryzae* (Ahlb.) Cohn:
    - Showing transient green: Takahashi's N, near *A. perniciosus.*
    - Showing no transient green: Takahashi's D, near *A. giganto-sulphureus.*
  - 2. Bridging forms intermediate in measurements between typical *A. flavus* and *A. oryzae*: *A. pseudoflavus* Saito. *A. gymnosardae* Yukawa.
  - 2. Bridging forms as no. 2 with much smaller conidia:
    - Takahashi's P, near *A. micro-virido-citrinus* Cost. and Lucet.
- *B.* Probably belonging with *A. tamari*:
  - Color butter-yellow, conidia 5 to 7 μ: *S. butyracea* Bainier.
  - Color umbrinus, conidia 6 to 9 μ, white sclerotia: *A. umbrinus* Patterson.
- *C.* Pathogenic to man:
  - Reported from human ear: *A. flavescens* Wreden.
  - Reported from skin lesions: *A. Tokelau* Wehmer.

The following is a bibliography of organisms referred to in this paper or whose relationship to these groups is suggested by the literature. The citations are arranged alphabetically to specific names.

S. albo-lutea Bainier. Bull. Soc. Bot. France **27**: 30. 1880. This was a small pale yellow form, but no further data were given and it has not since been identified.

A. aurantiacus Berkeley is cited by Montagne (Ann. Sci. Nat. Bot. III, **12**: 299. 1849) as having clavate heads. Farlow (Bibliographical Index of North American Fungi **1**, pt. 1, p. 276, issued Sept. 1, 1905) places this organism with *Nematogonium aurantiacum* Desm. Personal examination of the Curtis collection confirms Farlow's belief that this fungus is not an Aspergillus.

A. aureus Berkeley (Berkeley, M. J.). English Flora **5**: 346. 1836. The golden yellow, elliptical conidia reported by Berkeley suggest *A. citrisporus*, but actual identification from the description is impossible.

S. butyracea Bainier. Bull. Soc. Bot. France **27**: 29. 1880. C. Roumeguère. Fungi Gallici Exsiccati, no. 995. The organism has not since been reported. The material preserved in the Harvard herbarium shows some conidial fruiting fairly typical of *A. niger*, areas which correspond with Bainier's *S. fusca*, and some material which was probably *S. butyracea* but in which details of head structure, mature conidial markings, and measurements were not determinable with certainty. The information obtainable suggests relationship to *A. tamari*.

A. citrisporus von Höhnel. Fragmente zur Mykologie, I. Mittheilung. Sitzungsber. K. Akad. Wiss. Wien, Math.-Naturw. Kl. Abt. I, **111**: 987. 1902. This form was described by von Höhnel from excrement of larvae. A cultural description is given under this name to a form similarly isolated by Thaxter (no. 4186.10). A specimen was collected by Peck at Sandlake, N. Y., from excrement of caterpillars and preserved in the herbarium of the New York Botanical Garden. Another specimen of this species is found in the collection of the Michigan Academy of Science as determined by Kauffman.

S. castanea Patterson. Bull. Torrey Bot. Club **27**: 284. 1900. The color given suggests *A. tamari*, but the exsiccati show that this form was one of the paler forms of *A. niger*.

A. effusus Tiraboschi. Annali di Botanica **7**, fasc. 1: 16. 1908. Name cited without description by Tiraboschi in Atti Terzo Congresso Pellagralogico Italiano, Milano 1906: 139, 142. 1907. This name is accepted for a series of cultures obtained in America and described (page 109).

A. flavescens Wreden. Compt. Rend. Acad. Sci. Paris **65**: 368. 1867. Also St. Petersb. Med. Zeitschr. **13**: 133. 1867. There is no record that the organism of Wreden was cultivated. It was tentatively placed by us (Thom, C., and Church, M. B. Amer. Jour. Bot. **5**: 100. 1918) with *A. nidulans* from the descriptions given, but has been repeatedly cited as a synonym of *A. flavus*. The name was used by Lichtheim (Lichtheim, L. Ueber pathogenen Schimmelpilze. Berliner Klin. Wochenschr. **19**: 128, 147. 1882) and others for a strain certainly belonging to the *A. flavus* series. There is no direct evidence that this usage was based upon positive identification of Wreden's material.

A. flavus Link. Obs. p. 16. 1809. (Link, H. F. Observationes in ordines plantarum naturales. Ges. Naturforsch. Freunde zu Berlin, Magazin 3. 1809, usually cited Link Obs.). The habitat cited by Link was badly dried herbariun specimens. DeBary and Woronin in describing *Eurotium A. flavus* (DeBary, A., and Woronin M., Beiträge zur Morphologie und Physiologie der Pilze, III^{te} Reihe, 2te Abt., p. 380) believed their material to be identical with the species of Link. A culture by Brefeld preserved in Rabenhorst, Fungi Europaei Edit. Nov. ser. II, no. 2135, is cited by Wilhelm (Wilhelm, K. A. Beiträge zur Kenntniss der Pilzgattung Aspergillus. Inaug. Diss. Strassburg, p. 59. 1877), and later by Wehmer (Monog. p. 81. 1901). The continuity of the usage of the name *A. flavus* seems therefore well established. We have examined a packet of this material in the collection of the New York Botanical Garden, which is certainly the organism we have described as *A. flavus*. Costantin and Lucet (*loc. cit.*, pp. 162, 163) attribute the name *A. flavus* incorrectly to Wilhelm.

A. fulvus Montagne. Plantes cellulaires, Cent. VI, no. 82. Ann. Sci. Nat. Bot. III, **12**: 298. 1849. The description allies this form with the group typified by *A. tamari* in this paper, but *A. fulvus* has never been reported except by Montagne.

A. giganto-sulphureus Saito. Jour. Coll. Sci. Imp. Univ. Tokyo **18**: 48. Pl. 3, figs. 12*a-d*. 1904. While not identified by us positively, the description suggests colonies near D, in Takahashi's series.

A. gymnosardae Yukawa. Jour. Coll. Agr. Tokyo **1**: 362. Pl. 18, figs. 1–7. 1911. This fungus was found by Yukawa under the name "awokabi" and is described by him as essential to the ripening of the tunafish preparation, "katsuobushi." The dimensions given are intermediate between those of *A. flavus* and of *A. oryzae*, and closely approximate those of *A. pseudoflavus*. Although we have cultures related to these forms, we have not been able to identify these intermediates.

A. micro-virido-citrinus Costantin & Lucet. Ann. Sci. Nat. Bot. IX, **2**: 158. 1905. The appearances of colonies and measurements of stalks, heads, and spores indicate a form intermediate between *A. flavus* and *A. oryzae* except for its small conidia. The description is very nearly satisfied by Takahashi's culture P.

A. oryzae (Ahlburg) Cohn (Cohn, F. Ueber Schimmelpilze als Gärungserreger. Jahresb. Schles. Ges. für vaterl. Cultur (1883) **61**: 226. Breslau. 1884). Syn., *Eurotium oryzae* Ahlb. The name *E. oryzae* with an incomplete description for the saké organism was published by Korschelt (Korschelt, O. Ueber Saké, das alkoholische Getränk der Japaner. Dingler's Polytechnisches Jour. **230**: 330. 1878) as taken from a letter from "Herr Ahlburg."

A. oryzae var. *basidiferens* Costantin & Lucet. Ann. Sci. Nat. Bot. IX, **2**: 167. 1905. The describers found both secondary and primary sterigmata upon a culture received by them as *A. oryzae*. Without cultivating any other strain, they describe this form as a new variety. Although double sterigmata are not mentioned in Wehmer's description, all strains seen by us have double sterigmata at least under some conditions of culture. Hence the varietal name should be dropped.

Eurotium oryzae Ahlburg. See *A. oryzae* (Ahlb.) Cohn.

A. parasiticus Speare. Hawaiian Sugar Planters' Exp. Sta., Path. and

Physiol. Ser., Bull. **12**: 38. Pls. 3, 4. 1912. Speare found this form parasitic upon the mealy bug of sugar cane in Hawaii. The same form has also been found by us on mealy bugs from Demerara. Experiments, however, with known strains of the *A. flavus* group show that parasitism on the mealy bug is not confined to Speare's strain.

A. perniciosus Inui. Jour. Coll. Sci. Imp. Univ. Tokyo **15**: 473. 1901. Inui recorded a transient green phase in the colonies of this species which is otherwise compared to *A. luchuensis* and *A. Wentii.* In our cultures, *A. luchuensis* and *A. Wentii* have stalks smooth, not pitted as found by Inui in his form. The description suggests certain variant types among Takahashi's cultures such as N.

A. pseudoflavus Saito. Centralbl. Bakt. II, **18**: 34. figs. 15–18. 1907. Syn., *S. pseudoflava* Sacc. Sylloge Fungorum **22**: 1260–1266. The morphology given indicates that *A. pseudoflavus* is one of the intermediate forms which bridge the gap between typical *A. flavus* and *A. oryzae.*

A. siebenmanni Costantin & Lucet. Ann. Sci. Nat. Bot. IX, **2**: 162. 1905. This name is based upon Siebenmann's (Siebenmann, F. Die Fadenpilze *A. flavus, niger, fumigatus, Eurotium repens* und ihre Beziehung zur *Otomycosis aspergillina.* Zeitschr. f. Ohrenheilk. **12**. 1883. Die Schimmelmycosen des menschlichen Ohres. Wiesbaden, 1889) description of an organism from the human ear identified by Siebenmann as *A. flavus*, but regarded by the describers from the description given by Siebermann as a separate species. The data given place the organism correctly in the *A. flavus* group but are not complete enough to separate it.

A. tamari Kita. Centralbl. Bakt. II, **37**: 433. 1913. The strain described in the text was verified as *A. tamari* by Kita. Numerous strains of this group, some of which vary appreciably in cultural detail from the type, have been obtained from purely American as well as from Oriental sources.

A. terricola Marchal (Marchal, Émile). Rev. Myc. **15**: 101. 1893. See also Scales, F. M. Jour. Biol. Chem. **19**: 459. 1914. The culture isolated by Scales was sent by us to Marchal and designated by him *A. terricola* var. *Americana* Marchal in this paper.

A. terricola var. **Americana** Marchal n. var. cultural description Thom and Church. Colonies on Czapek's solution agar from shades near *yellow ocher* (Ridgway XV. 17) when young to *Dresden brown* or *mummy brown* of the same plate (near Saccardo's umbrinus); aerial growth consisting of crowded conidiophores, stalks 300 to 600 μ by 6 to 8 μ, walls pitted; heads radiate; vesicles up to 20 μ in diameter; sterigmata in one series, 7 to 10 μ by 2 to 4 μ; conidia tuberculate from the presence of color bars variously distributed between the outer and inner wall, ovate, from 3 by 5 μ up to 5 by 7 μ or nearly globose, usually about 5.5 μ, occasionally 5 to 8 μ in diameter. Culture by F. M. Scales from Georgia soil. This variety "differs from the type in measurements of vesicle 14 to 20 μ in diameter instead of 40 to 50 μ, in sterigmata 5.6 to 10.5 μ by 2.2 μ instead of 12 to 15 μ by 4 to 7 μ" (Marchal).

A. tokelau Wehmer (Wehmer, C.). Centralbl. Bakt. I, **35**: 140. 1903. The measurements given by Wehmer together with the figures given by Dubreuihl (Dubreuihl, M. W. Jour. Méd. Bordeaux **32**: 312. 1902.) suggest relationship with the *A. flavus* group.

A. umbrinus Patterson (Patterson, Flora W. Bull. Torrey Bot. Club **27**: 284. 1900). The original material of *A. umbrinus* appears to be lost. Although probably related to *A. Wentii* or to *A. tamari*, no material identifiable by this description has been seen.

A. variabilis Gasperini (Gasperini, G. Atti Soc. Toscana Nat. Sci. Pisa, Mem. 8, fasc. 2: 326. 1887). From the description, this was probably some strain of the *A. flavus* group. Both large and small heads were found in the same colony; the sterigmata were simple or double; the conidia have the range of form and size characteristic of the group. Gasperini does not appear to have known *A. flavus*.

A. Wehmeri Costantin & Lucet. Ann. Sci. Nat. Bot. IX, **2**: 162. 1905. The name is proposed by Costantin and Lucet for the organism of Brefeld and Wehmer described as *A. flavus* Link by them and so used in this paper. The uncertainties in the identification of Link's species do not seem important enough to justify the change of name. *A. Wehmeri* is to be regarded as a synonym of *A. flavus*.

A. Wentii Wehmer. Centralbl. Bakt. II, **2**: 150. 1895. See also Wehmer, Die Pilzgattung Aspergillus, etc., in Mém. Soc. Phys. d'Hist. Nat. Genève **33**: 2. Part. 4: 119. 1901. The culture described in the text was received from Amsterdam and appears to be the original strain investigated by Wehmer.

Announcing the publication of

The Chemistry of Plant Life

By ROSCOE W. THATCHER

Dean of the Department of Agriculture, University of Minnesota

McGraw-Hill Agricultural and Biological Publications

268 pages, 6 x 9, $3.00 net, postpaid

THE first American textbook devoted to the chemistry of plant cell activities. The material is the development of the course in Phyto-chemistry offered by the author for several years to the students of the Plant Science Group of the University of Minnesota. The book aims to furnish a proper foundation upon which to build a scientific knowledge of how plants grow.

Send for a copy of this new book on approval

McGraw-Hill Book Co., Inc.
Penn Terminal Building
370 Seventh Avenue New York

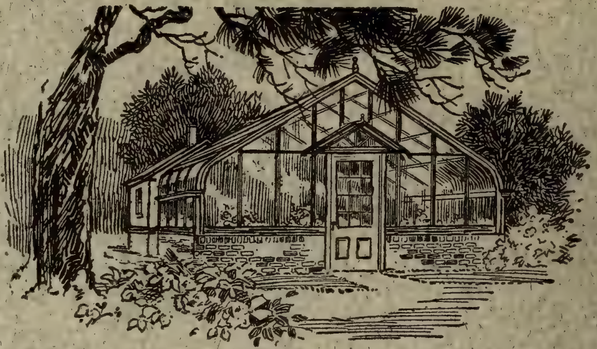
PHOTOMICROGRAPHS

HIGH GRADE RESULTS MADE FROM YOUR MATERIAL
BLACK AND WHITE OR DIRECT COLOR PHOTOS
ORDINARY OR POLARIZED LIGHT
LANTERN SLIDES OR PRINTS FURNISHED

PLAIN OR HAND-COLORED SLIDES OF BOTANICAL SUBJECTS FOR SALE

Ask for subjects desired

ARTHUR G. ELDREDGE
Box 83, Univ. Sta. Urbana, Illinois



Do Our Greenhouses Really Differ So Much From Others

Outwardly most folks are mostly alike.

So are most greenhouses.

But inwardly—that's quite a different matter.

Just as the way a man gives attention to the little things—tells you the kind of a man he is; just so the care and particularity given to the little details in a greenhouse, tells you the kind of a house it is.

Some owners have accused us of being rather fussy about our work, intimating that we could cut corners and get the work done quicker.

And they are right.

But you'll agree that we are also right in refusing to do it.

That then might be one of the differences in which our greenhouses differ.

Hitchings and Company

Home Office and Factory: ELIZABETH, N. J.

NEW YORK
101 Park Ave.

BOSTON-9
294 Washington St.

Vol. VIII MARCH, 1921 No. 3

AMERICAN JOURNAL OF BOTANY

OFFICIAL PUBLICATION OF THE
BOTANICAL SOCIETY OF AMERICA

4 1921
ional Museu

CONTENTS

A study of Rhus diversiloba with special reference to its toxicity James B. McNair 127

The effect of salt proportions and concentrations on the growth of Aspergillus niger . . . . C. M. Haenseler 147

Suggestions with respect to the measurement of osmotic pressure L. Knudson and S. Ginsburg 164

Thick-walled root hairs of Gleditsia and related genera W. B. McDougall 171

A simple method for growing plants . . . . J. M. Brannon 176

PUBLISHED
IN COOPERATION WITH THE BOTANICAL SOCIETY OF AMERICA
At 41 North Queen Street, Lancaster, Pa.
BY THE
BROOKLYN BOTANIC GARDEN
Brooklyn, N. Y.

Entered as second-class matter February 21, 1914, at the post office at Lancaster, Pennsylvania, under the act of March 3, 1879

AMERICAN JOURNAL OF BOTANY

DEVOTED TO ALL BRANCHES OF BOTANICAL SCIENCE

ESTABLISHED 1914

EDITED BY A COMMITTEE OF THE BOTANICAL SOCIETY OF AMERICA

EDITORIAL COMMITTEE

C. E. ALLEN, Editor-in-Chief,
University of Wisconsin

WILLIAM CROKER,
University of Chicago

L. R. JONES,
University of Wisconsin

C. STUART GAGER, Business Manager
Brooklyn Botanic Garden

ORLAND E. WHITE,
Brooklyn Botanic Garden

R. A. HARPER,
Columbia University

JACOB R. SCHRAMM,
Cornell University

C. L. SHEAR, *Bureau of Plant Industry*
(Representing American Phytopathological Society)

The Journal is published monthly, except during August and September. Subscription price, $6.00 a year. Single copies 75 cents. Back numbers, 75 cents each; $6.00 a volume, postage extra. Postage will be charged to all foreign countries, except Mexico, Cuba, Porto Rico, Panama Canal Zone, Republic of Panama, Hawaii, Philippine Islands, Guam, Samoan Islands, and Shanghai. Postage to Canada, 20 cents a volume on annual subscriptions; to all other countries in the Postal Union, 40 cents a volume on annual subscriptions.

The pages of the Journal are open to members of the Botanical Society of America, or to candidates approved for membership.

Manuscript offered for publication should be typewritten, and should in all cases be submitted to the Editor-in-Chief.

Papers are limited to 20 pages in length, exclusive of plates, except that additional pages may be arranged for at cost rate to authors (approximately $3.00 a page).

Proofs should be corrected immediately on receipt, and returned to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y.

Illustrations, other than zinc etchings, and tabular matter should, in general, be restricted to approximately ten percent of the number of pages of text. Authors may be charged at cost rates for illustrations and tabular matter in excess of that amount (approximately $6.40 a page for tabular matter; 15 cents per square inch for half-tones).

Separates should be ordered when proof is returned. Ffty copies without cover will be supplied free; cover and additional copies at cost.

Remittances should be made payable to American Journal of Botany. Ten cents must be added to all checks not drawn on New York City banks.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

Correspondence concerning editorial matters should be addressed to Prof. C. E. Allen, University of Wisconsin, Madison, Wisconsin.

Business correspondence. including notice of change of address, and directions concerning reprints, should be addressed to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y., or 41 North Queen Street, Lancaster, Pa.

AMERICAN
JOURNAL OF BOTANY

VOL. VIII MARCH, 1921 NO. 3

A STUDY OF RHUS DIVERSILOBA WITH SPECIAL REFERENCE TO ITS TOXICITY

JAMES B. McNAIR

(Received for publication September 15, 1920)

Rhus Toxicodendron (L.), *Rhus radicans* (L.), and *Rhus diversiloba* T. & G. form a triad of plants equally regarded with aversion. The general recognition of their deleterious character is evinced in the application of the names *poison ivy*, *poison vine*, and *poison oak*, given to them in various parts of the United States.

Perhaps the earliest mention of these plants in North America is the following description by Captain John Smith in 1609:

> The poisonous weed, being in shape but little different from our English yvie; but being touched causeth reddness, itchinge, and lastly blysters, the which, howsoever, after a while they passe awaye of themselves without further harme; yet because for the time they are somewhat painefull, and in aspect dangerous, it hath gotten itselfe an ill name, although questionlesse of noe very ill nature.

Long before the birth of Linnaeus, Cornutus in 1635 described the plant as a species of ivy in his work on the plants of Canada (*Hedera trifolia Canadensis* Corn. 96 from Carolina in the British Herbarium).

About 1736 Linnaeus classified this plant as *Toxicodendron triphyllum glabrum*. At the same time he described and named *Rhus radicans*.

In an entry dated October 9, 1748, Peter Kalm gave an extensive and interesting description in his travels in North America of the *Rhus radicans* of Linnaeus. Since that time there have been many accounts of these plants and of their toxic nature.

In 1820, Bigelow described *Rhus radicans* of Linnaeus as having

> Ternate leaves, that grow on long semicylindrical petioles. Leaflets ovate or rhomboidal, acute, smooth and shining on both sides, and veins sometimes a little hairy beneath. The margin is sometimes entire and sometimes variously toothed and lobed, in the same plant. The flowers are small and greenish white. They grow in panicles or compound racemes on the sides of the new shoots and are chiefly axillary. The barren [male] flowers have a calyx of five erect, acute segments, and a corolla of five oblong recurved petals. Stamens erect with oblong anthers. In the center is a rudiment of a style. The fertile [female] flowers situated on a different plant, are about half the size of the preceding. The calyx and corolla are similar but more erect. They have five small, abortive stamens and a roundish germ [ovule] surmounted with a short, erect style ending in three stigmas. The berries are roundish and of a pale green color, approaching to white.

[The **Journal** for February (**8**: 59–126) was issued March 19, 1921.]

> A plant has long appeared in the Pharmacopoeias under the name of *Rhus Toxicodendron.* Botanists are not agreed whether this plant is a separate species from the one under consideration, or whether they are varieties of the same. Linnaeus made them different with the distinction of the leaves being naked and entire in *Rhus radicans*, while they are pubescent and angular in *Rhus Toxicodendron.* Michaux and Pursh whose opportunities of observation have been more extensive, consider the two as mere local varieties; while Elliott and Nuttall still hold them to be distinct species. Among the plants which grow abundantly around Boston, I have frequently observed individual shoots from the same stock having the characters of both varieties. I have also observed that young plants of *Rhus radicans* frequently do not put out rooting fibers until they are several years old and that they seem, in this respect, to be considerably influenced by the contiguity of supporting objects.

The attitude taken by Bigelow has been sustained by later botanists, among them Torrey and Gray (58) who consider *R. radicans* a variety of *R. Toxicodendron.*

Rhus diversiloba was first discovered by Douglas at Fort Vancouver on the Columbia river about 1830. Upon examination of this specimen W. J. Hooker (26), although he considered it "nearly allied, as this assuredly is, to the two preceding species [*R. Toxicondendron* and *R. radicans*]," nevertheless "ventured to consider it distinct." He therefore gave it botanical significance as *Rhus lobata.* To support his conclusion he advances the following reasons:

> It's general habit is very different, having erect straight *stems* and numerous small leafy *branches.* The leaflets besides being deeply lobed with acute sinuses are truly ovate, very obtuse, and greatly smaller than in any state of *R. Toxicodendron*, or *R. radicans*, which I have seen; the *panicles*, too, are exceedingly numerous.

A free translation of Hooker's Latin description of the plant is as follows:

Bush erect, 3–4 feet, branches round with the youngest ones pubescent, branches numerous, short, spreading, leafy. *Leaves* long-petiolate, trifoliate, with little leaves ovate, 1–2 inches long, very obtuse, membranaceous, at the base sometimes acute, sometimes rotund or truncate, beneath especially pubescent, deeply and variously lobate, terminate one sub-long-petiolate, each side sub-equally lobate with lobes generally less than 3, with little lateral leaves at the exterior margin more deeply lobate. *Flowers* (male) yellow, in loose racemes, shorter than leaf, longer than petiole. *Bracts* at the base of the branches oblong, ciliate. *Calyx* deeply parted with oblong lappets. *Petals* 5, much longer than the lappets of the calyx, obovate into a tongue evidently with attenuated base, at the back veined. *Stamens* 5, erect, little shorter than petals. *Filaments* subulate. *Anthers* 5, somewhat more greatly ovate, pale yellow, with cells sub-opposite. *Style* small, extending from the center of a platter-shaped disc situated in the bottom of the calyx, margin of the disc elevated, curled.

The next known discovery of *R. lobata* was that of Capt. Beechy (Hooker and Arnott, 27) at San Francisco and Monterey Bay about 1832. These specimens differed in no respect from the more northern ones discovered by Mr. Douglas.

The observations of Nuttall (Torrey and Gray, 58) furthered the botanical knowledge of the plant. He noticed that

Comparison of Flowers of Rhus Toxicodendron (Poison Ivy) and Rhus diversiloba (Poison Oak)

	Male		Female	
	Rhus diversiloba	*Rhus Toxicodendron*	*Rhus diversiloba*	*Rhus Toxicodendron*
Panicles:				
Color	Light green	Light green	Light green	Light green
Number	As many as leaves on flowering shoot, except none in highest or lowest axils	As many as leaves on flowering shoot, except none in highest or lowest axils.	$\frac{1}{2}$ as many as leaves. Total number about the same as in male (3 to 5)	
Length	7 cm.	3–5 cm.	3–6 cm.	3–4 cm.
Angle with stem	Sharp. 45°	Sharp. 45°	Obtuse, limp.	Obtuse, limp.
Number of side twigs of first order	12	12	12	5
Length	3 lowest, 2 cm.	3 lowest, 2 cm.	2.5 cm.	1.5–2 cm.
Phyllotaxy	Same as leaf	Same as leaf (3/9)		
Flowers:				
Number				
Pedicel length	4–7 mm.	2–3 mm.	5–10 mm.	1.5–2 mm.
Flower width	5-7 mm.	9 mm.	5 mm.	4 mm.
Calyx leaves:				
Number	5	5	5	5
Length	2 mm.	2 mm.	2 mm.	1 mm.
Width		1 mm.		
Shape	Tongue-shaped	Tongue-shaped	Tongue-shaped	Tongue-shaped
Color	Dark green	Dark green	Dark green	Dark green
Petals:				
Number	5	5	5	5
Length	4 mm.	4 mm.	3 mm.	2 mm.
Width	1.5 mm.	2 mm.	1.5 mm.	1 mm.
Shape	Elliptical, curved down	Elliptical, curved down	Not curved down as much as male	Not curved down as much as male
Color	Light green	Light green		
Stamens:				
Number	5	5	5	5
Length	2.5 mm.		1.5 mm.	
Anther:				
Variety	Introrse, shrunken	Introrse	Introrse, shrunken	Introrse, shrunken
Filament:	Dirty yellow color		Dirty yellow color	Dirty yellow color
Length	2 times as long as anther			

Comparison of Flowers of Rhus Toxicodendron (Poison Ivy) and Rhus diversiloba (Poison Oak)—Continued

	Male		Female	
	Rhus diversiloba	*Rhus Toxicodendron*	*Rhus diversiloba*	*Rhus Toxicodendron*
Pollen:				
Size	1/800 sq. mm. in horizontal area			
Shape	Wide 1/3–1/2 of length			
Color	Yellow		Absent	
Condition	Rough with sharp-pointed cells, adhesive			
Ovules:				
Number				
Size	1 mm. high			
Shape	Keg		Egg-shaped	
Color				
Condition	Rudimentary	Rudimentary	Fully developed	Fully developed
Carpels			3, of which 2 are rudimentary	3, of which 2 are rudimentary
Stigmas:				
Number	3			
Size				
Shape				
Color				
Condition				

Specimens of *R. Toxicodendron* collected in the Botanical Garden of the University of Pennsylvania.

Specimens of *R. diversiloba* collected at Berkeley, California.

> The sterile and fertile flowers of this species (which is very near *R. Toxicodendron*) present some notable differences. The sterile, which is figured by Hooker, has rather deeply lobed leaflets, sometimes in fives and larger flowers; in the fertile the leaflets are almost entire or slightly lobed and the flowers considerably smaller, so that it might readily be taken for a distinct species. The fruit is white, somewhat pubescent and gibbous.

Torrey and Gray (58) summed up the previous knowledge of the plant and renamed it *Rhus diversiloba*, the name by which it is now more commonly known.

The difference between *R. diversiloba* and *R. Toxicodendron* is so small that their proper classification forms a bone of contention between botanists. Those botanists who believe in innumerable species are in favor of their separation, while the more conservative are opposed to it. Greene (21) considers *R. diversiloba* "a peculiar type of *Toxicodendron* belonging exclusively to the Pacific Coast." Engler (15) believes *diversiloba* a subspecies of *Toxicodendron*. The only botanical ground for the separation of the two into different species is a slight difference in the shape of their leaflets (Gray, 17). A three years' study of *Rhus diversiloba* and a recent study of *R. Toxicodendron* in Pennsylvania and Maryland for a year have enabled me to make a personal comparison of the two plants. The tracings of the outlines of mature leaves of both plants (figs. 1, 2) and a tabular account

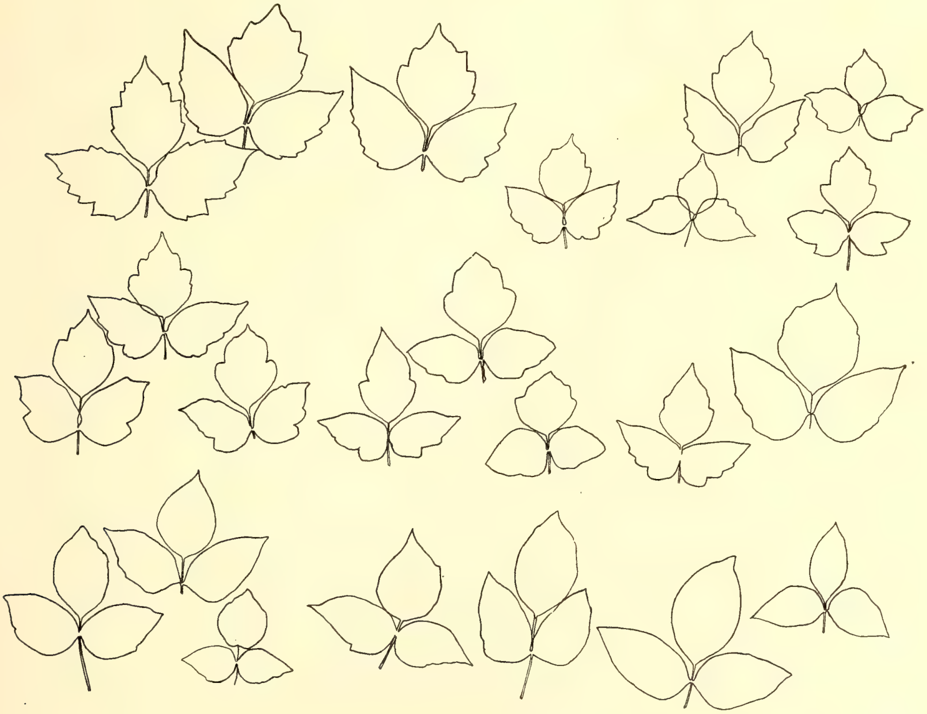


FIG. 1. Tracings of mature leaves of *Rhus Toxicodendron* (Reduced 6¾×).

of the flowers of both plants will permit the reader to decide whether or not there is sufficient difference to constitute a separation into species.

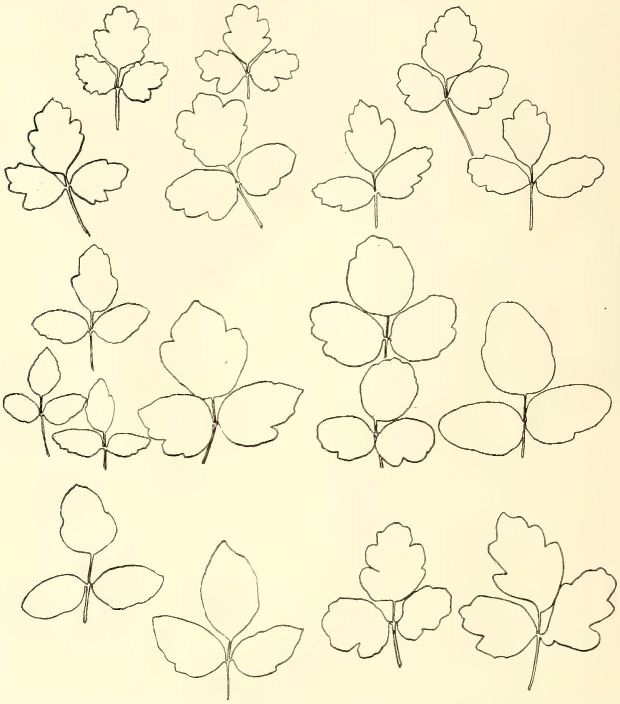


FIG. 2. Tracings of mature leaves of *Rhus diversiloba* (Reduced 6¾ ×).

GEOGRAPHICAL DISTRIBUTION OF R. DIVERSILOBA

The distribution of poison oak includes Lower California north of latitude 29° (Brandegee, 6), Santa Barbara and Santa Catalina Islands (Brandegee, 7), California, Oregon, Washington, Vancouver Island, and British Columbia. The region inhabited by the plant has been approximately defined by citations from botanical literature, sources of herbarium specimens, and places where birds were found that had poison oak seeds in their stomachs. From these data, the territory inhabited by poison oak embraces the Sonoran and lower transition life zones, and excludes the desert and central valley regions of California together with the upper transition and boreal zones.

The inhabited area has an altitude varying from sea level to 6,000 feet above sea level. Hall's (24) observations in the Yosemite Valley make it an inhabitant of the Hetch Hetchy and the low foothills with a maximum altitude of about 4,000 feet. In southern California he noticed it in the San Jacinto Mountains along the North Fork of the San Jacinto river at an altitude of approximately 3,000 feet. I have found it in Cold Water Canyon on the southwest side of Mt. San Antonio in the San Gabriel Range in southern California at an altitude of 4,500 feet. The lowest and highest regions of California are therefore free from poison oak.

From rainfall data compiled by the United States Government the plant requires an annual rainfall of at least ten inches.

Geographical Distribution of Rhus diversiloba (Poison Oak) According to Literature aud Correspondence

Date	Author	Location
1831	Douglas	Common on the outskirts of woods in dry soils in northwest America. Plentiful at Fort Vancouver.
1832	Hooker and Arnott	San Francisco and Monterey Bay.
1845	Lindley and Lyon	Common everywhere in California. An inhabitant of Santa Catalina Island.
1855	Newberry	Common throughout northern California; more rare in the Klamath Basin.
1856	Torrey	Plains and mountains near San Gabriel; Martinez.
1876	Brewer and Watson	From southern California to British Columbia; in California most abundant in the Coast Range.
1878	Wheeler	Common on the Pacific Coast.
1886	Greene	On the north side of Santa Cruz Island.
1886	Lyon	An inhabitant of Santa Catalina Island.
1889	Brandegee	In Lower California, very abundant about El Rosario.
1890	Brandegee	Common on San Miguel, Santa Rosa, Santa Cruz, and Santa Catalina Island.
1893	Coville	Found at several points on rocky hillsides in the foothill belt of the western slopes of the Sierra Nevada.
1894	Greene	Copious in the Coast Range hills, preferring cool northward slopes and the banks of streams; absent from the more elevated portions of the Sierra.
1895	Gray	Common throughout California, north to the borders of Washington.
1897	Jepson	Fort Bragg to Sherwood Valley in redwood belt.
1898	Howell	In forests and rocky hillsides, British Columbia to California.
1898	Jepson	Mitchell Canyon, Mount Diablo.
1899	Jepson	Crane Creek and Rosewood, Stiver's ranch.
1900	Jepson	Cedar Creek.
1901	Jepson	Smith Mountains, Palomar, 6,000 feet.
1901	Jepson	Kaweah Range, north slopes and moist places.
1901	Jepson	St. Helena, climbing redwood.
1901	Jepson	Pine Canyon, Mt. Diablo, shrubs 6 feet high.
1902	Chestnut	Common in valleys and on hillsides everywhere throughout Mendocino County.
1902	Jepson	Givin Mine, Calaveras County, 1,100 feet altitude. Shrubs 12 and 13 feet high. Schoolhouse Creek, Ft. Bragg, Cahto. Redstone Park. Hawley School, Willits. Fort Seward, Ranch Ridge, Humboldt County, 3,000 feet. Abundant. Idolwild (near Camp Grant), Humboldt County. Climbs up redwood trunks 90–100 feet. Hawkins Bar (Dyer's ranch). Usal to Cottonaby Creek.

1903	Greene	A peculiar type of *Toxicodendron* belonging exclusively to the Pacific Coast.
1903	Jepson	Along fences in Vacaville. Vacaville to Twin Sister's Peak. Vacaville Rock Peak.
1906	Piper	Washington to California in the coast regions. Humid transition zone.
1907	Jepson	Cudahay Trail to Dutch Henry's on Klamath River, 2,500–4,000 feet, below fir zone, only along river.
1909	Parsons	Throughout California, save in the high Sierras.
1909	Jepson	Pepperwood, Humboldt County, in redwood trees. Hetch Hetchy (3,700 feet).
1910	Jepson	Belden, 2,000 (approximate) feet. Half Moon Bay.
1911	Jepson	Arroyo Seco, Monterey County, altitude 100–500 feet. Napa Range near Atlas Peak.
1911	Abrams	Frequent in chaparral belt throughout southern California.
1911	Muir	Common throughout the foothill region up to a height of at least 3,000 feet above sea level.
1911	Jepson	Found in Coast Range and foothills of the Sierra Nevada, widely distributed and often abundant.
1912	Hall	Confined to lower end of Yosemite Valley, and to the Hetch Hetchy and low foothills.
1912	Jepson	Nelson, middle Tule River, altitude 4,760 feet. Saratoga, Santa Clara County, altitude 600 feet.
1916	Hall	Merced Canyon, not rare to 3,200 feet altitude. West of Wawona at 4,500 feet. Small. In Santa Cruz Mountains, dominant shrub Alma to summit, especially in redwood belt.
1916	Sanborn	Abundant in the hills about Eugene, and all through the western part of Oregon.
1916	Crawford	In mountain canyons and valleys from sea level to about 7,000–7,500 feet elevation. Very common throughout Pomona Valley and all the valley regions between San Bernardino and the coast.
1916	Parish	Grows to some extent in damp soil in San Bernardino Valley, altitude 1,000 feet, and abundantly in the canyons of the southern slope of the San Bernardino Mountains up to 3,500 feet altitude at least. Does not grow in the higher mountains nor in either the Mojave or the Colorado Desert.
1919	Jepson	Dunsmuir to Castle Rock Station along Sacramento River, 2,200 feet altitude.

Locations where Birds which had eaten Rhus diversiloba Fruit were Collected

CALIFORNIA

Alhambra
Arroyo Valley Creek
Berkeley
Berryessa
Camp Meeker
Chico, Tehama County
Claremont
Cull Canyon
Guadalupe
Pinte Mountains
Rio Dell, 15 miles southwest
San Antonio Canyon
San Fernando
San Jose
Santa Clara County
Santa Monica Mountains
Santa Rosa
Sierra Morena, 6 miles

Haywards
Mt. Diablo
Northwest of Pasadena
Palo Alto
Pasadena
Payne P. O., Tehama County
Petrolia
Simol
Smith Creek
South of Palo Alto
Stewart's Ranch
Voltas
Watsonville

OREGON

Ashland
Bybee's Bridge
Coquille
Los Gatos

WASHINGTON

Garfield County

(The above list was communicated to me by Dr. E. W. Nelson, Acting Chief, Biological Survey, U. S. Department of Agriculture.)

Distribution of Rhus diversiloba According to Sources of Herbarium Specimens

CALIFORNIA

Alpine, San Diego County, Mearns 4019.
Alum Rock Springs, vicinity, Santa Clara County.
Big Chico Creek Canyon, Butte County, altitude 250 feet, A. A. Heller.
Black Mountain, Santa Clara County, Elmer 4785.
Blochman's Ranch, Mariposa County, Alice Eastwood.
Cantara, Siskiyou County, Alice Eastwood.
Carmel, Monterey County.
Casitas Pass, Ventura County, altitude 1000 feet.
Chico, near, Palmer 2060.
Clayton, Contra Costa County, Brewer 1068.
Cow Creek Mts., Shasta County.
Folsom.
Forest Ranch, 1897, Mrs. R. M. Austin 1801.
Fort Tejon, vicinity, Kern County.
Foster Park, Ventura County, Alice Eastwood.
Gasquet, Del Norte County, Alice Eastwood.
Havilah, Grinnell 362.
Healdsburg, Sonoma County.
Kings Canyon, Lieber Mts., Los Angeles County.
Little Chico Creek, Austin 749.
Los Gatos foothills, 1904, A. A. Heller 7327.
Los Tronus Creek, San Mateo County.
Mendocino, near, H. E. Brown 750.
Monterey, Bailey.
Monterey, Botta in Mus. Herb., Paris.
Mount Diablo, Alice Eastwood.
Mutair Flat, Ventura County.
New York Falls, Amador County, altitude 2000 feet.
North Fork and vicinity, Griffiths 4531.
Oroville, Table Mt., 8 miles north of, Butte County.
Pacific Grove.
Petrified Forest, Alice Eastwood.
Palo Alto, foothills near, Santa Clara County.
Pasadena, Jones 3206.
Red Reed Canyon, Ventura County.

Round Valley, Mendocino County.
St. Helena, vicinity.
San Clemente Island, Mearns 4048.
San Diego, canyons near, J. J. Hernleer.
San Francisco, Lone Mt. Cemetery.
San Franciquito Creek, San Mateo County.
San Jacinto Mts., shade along north fork of San Jacinto River, altitude 3000 feet, H. M. Hall.
Santa Barbara, Elmer 3940.
Santa Clara County, J. J. Hernleer.
Santa Cruz, Marcus E. Jones.
Santa Cruz Island, Stanford Herbarium.
Santa Cruz Mountains, 1903, N. L. Gardner.
Sausalito Hills, Kellogg and Herford 332.
Savage Hill, Amador County, altitude 2200 feet, Hansen 53.
Shasta River, near mouth, Siskiyou County.
Stanford University, Santa Clara County, Rutter 163.
Stanford foothills, Baker's collection no. 547.
Sulphur Banks, Lake County.
Sulphur Mountain Spring, Sulphur Mountains, Abrams and McGregor 46.
Sulphur Mt. Spring, Ventura County.
Table Mt., Butte County, altitude 600 feet.
Tamalpais.
Tassajara Hot Springs, Elmer 3178.
Topsajoin (?) Hot Springs, Monterey Co.
Vaca Valley, Solano County.

OREGON

Ashland, Stanford Herbarium.
Azalea Creek, Mears.
Cascade Mts., Moseley in Kew Herbarium.
Columbia River, between 46° and 49° latitude.
Columbia River, rocky places, Ethel I. Sanborn.
Coos Bay, House 4746.
Corvallis, 1898, Moses Craig.
Dallas.
Deschutes River, 1885, Thomas Howell.
Jackson County, along Walker Creek, altitude 3300 feet, Applegate 2339.
Lyall in Kew Herbarium.
Portland, 1885, L. E. Henderson.
Portland, open hillsides, Ethel I. Sanborn.
Portland, Walpole 44 and 8.
Portland, rocky hillsides, 1903, J. Lunell.
Rocky Butte, Multnomah County, Ethel I. Sanborn.
Umpqua Divide, head of Elk Creek, altitude 1500 feet, Leiberg 4190.
Umpqua River, east fork of North Fork, 6–10 miles east of Peel, altitude 1500 feet, Applegate 2700.
Umpqua-Rogue River Divide, dry hillsides, Ethel I. Sanborn.
Wasco County, 1896, L. F. Henderson.

WASHINGTON

American Lake, south of Tacoma, F. S. Hall.
Orchard Point, Kitsap County, F. L. Pickett.
Seattle, F. L. Pickett.

Seattle, F. S. Hall.
Tacoma, seashore and bluffs, F. L. Pickett.
Union City, F. L. Pickett.

VANCOUVER ISLAND

Vancouver Island, Tolmic, Douglas in Kew Herbarium.
Victoria, near Swan Lake and on the west side of Seanich Arm, J. R. Anderson.

THE ORIGIN AND OCCURRENCE OF THE POISON

The freshly exuded resinous sap of *R. diversiloba* has long been known to be capable of producing dermatitis when applied to the skin. With this in mind, investigations were carried out to see whether the poisonous portions of the plant are limited to those portions that contain the resin canals.

Microscopical examination of the staminate flower shows four resin ducts in the receptacle and pedicel, one in each petal, but no resin ducts more than half-way up the basal filaments of the stamens. Realizing the absence of resin canals in the anthers, it was thought perhaps the pollen might be non-toxic. (See Pl. II, *D*.) The pollen was collected by shaking the flowers over a glass funnel to the stem of which a test tube was attached. This pollen was found to be non-poisonous when rubbed into the skin of an individual sensitive to the poison. An alcoholic extract of the pollen was non-toxic, nor did the pollen or the alcoholic solution assume a dark brown color when treated for five minutes with potassium hydroxide as does the poison. It is concluded, therefore, that the pollen is incapable of producing dermatitis. Similar non-toxic results have been obtained with the pollen of *Rhus vernicifera* by Inui (30), with that of *R. Vernix* by Warren (59), and with that of *R. Toxicodendron* by Rost and Gilg (50).

C. Schwalbe (51) considered the poison of *R. diversiloba* to be excreted from glandular hairs on the surface of the plant. As the resin canals are not connected with the epidermis or with the trichomes, it was considered that these like the stamens might also be non-toxic. Two different forms of trichomes have been noticed on the plant, similar morphologically to those found by Möbius (42) on *R. vernicifera* and by Rost and Gilg (50) on *R. Toxicodendron;* namely, a unicellular or multicellular needle-shaped hair, and a multicellular club-shaped hair (Pl. II, *F*). Morphologically the club-shaped hairs seem to be glandular; first, the upper multicellular portion is sharply marked off from the basal portion, which resembles a stalk; second, the upper portion has thinner walls than the basal portion; third, they are found mostly on the young, rapidly growing organs of the plant, especially on the floral region and the leaves, less on the green stems, and hardly at all on the woody portions.

When the green stem, pedicel, or main ribs of the leaf, which are covered with trichomes, are rubbed on skin sensitive to the poison, no dermatitis results. Care must be taken, however, that the epidermis of the plant is not broken severely enough to cause the resinous sap to exude.

The fresh green leaves were placed in a finger bowl and soaked in room temperature in 95 percent alcohol for ten minutes. The leaves had been examined first under a hand lens to make sure that through possible injury no resinous sap was on the surface. When placed in the finger bowl the sap was prevented from running down the pedicel from the cut end into the alcohol. The leaves when taken out of the alcohol had lost their gloss. The pale yellowish alcoholic solution remaining was concentrated by boiling in an open beaker. It was found to be non-toxic. It was not darkened by potassium hydroxide nor did it respond to other chemical tests for the poison. These results indicate that neither the plant trichomes nor their exudate are poisonous.

The cork cells of the older stem were likewise found to be non-toxic either when the branch was rubbed on the skin or when an alcoholic solution was made of scrapings from the outermost cork cells of a branch as thick as a man's wrist.

As no resin ducts were seen on a microscopical examination of the pith of a one-year-old stem of the poison oak nor in the woody stem, experiments were undertaken to determine their toxicity. The bark was carefully removed from the pith, a clean knife being used to shave off the outermost portions of the pith. The pith was then cut up in small portions and extracted in a Soxhlet apparatus with hot 95 percent alcohol. This alcoholic solution when concentrated gave neither a physiological test for the poison nor any of the chemical tests.

A similar experiment carried on with the woody xylem gave corresponding results.

SUMMARY

1. The fresh sap emulsion is the only part of the plant capable of producing dermatitis.

2. Those portions of the plant that do not contain the resin canals do not normally have this kind of toxic effect.

3. The non-toxic portions are the anthers, pollen, xylem, epidermis, cork cells, and trichomes.

LIABILITY TO POISONING RELATIVE TO GROWTH OF PLANT

At just what stage in its life the *Rhus diversiloba* plant first contains its irritant poison has not yet been determined. After the plant has become several years old, however, all parts except the xylem, cork cells, epidermis, and trichomes are toxic. Although many persons know the sap of the stems and leaves to be poisonous, yet there are some who do not consider the sap of the roots toxic. Such is the case, however, as is attested by persons who have come in accidental contact with the broken roots of the plant while digging out other botanical specimens (Kunze, 35; Stirling, 55). The poisonous action of the roots might be expected from their structure, as they have numerous vertical resin canals encircling the xylem (Pl. II, *C*).

The resinous sap of the stems and roots retains its toxicity probably without much variation in amount or in the degree of virulency throughout the year. This is evinced not only by citations from literature (White, 61; Beringer, 3) and by statistics (table 1), but also by experiments conducted with the sap by the writer.

The virulency (the liability to cause poisoning) of the plant varies with the different seasons of the year in accordance with the stage of growth of the leaves, stems, and flowers. When the first leaves of the plant are unfolding in the spring they are very turgescent and easily injured. Analogously, the growing stems are less resistant than the mature stems. The mature leaves of the plant are not nearly as easily injured. Of the mature leaves, those that grow in the shade have a weaker structure than those which develop in the sun. From this fact one might expect the shade leaves to be less resistant to injury.

The amount of poison in the plant varies with the capacity of its resin canals. Of this variation in amount, that of the stems and leaves is most commonly effective in the index of virulency. The leaf area undoubtedly makes its greatest increase in spring between the time when the leaves begin to unfold and the time when the flowers open. From this latter time the leaf area of the plant is nearly constant until the leaves begin to fall in autumn. Four weeks are generally required for the full development of a leaf. The flower and leaf buds begin to expand simultaneously, but the leaves soon expand more rapidly and reach maturity before the flowers open (Pl. II, *B*). The staminate and pistillate plants begin to bloom at about the same time. At Berkeley, California, but few of the flowers were open April 4, 1915. The next spring the plants near the Greek Theatre, at Berkeley, bloomed mostly between March 22 and May 1. Either the amount or the virulence of the poison in the autumn leaves is less than that of the normal mature leaves. Of the autumnal leaves the red are less toxic than the yellow, and when the leaves have finally withered and fallen they are non-toxic (McNair, 40).

Inui (30) has noticed that the amount of secretion of *R. vernicifera* is influenced by the conditions of light and atmospheric humidity. In potted plants the secretion lessened when carbon assimilation was hindered. Similarly, secretion was greater in damp than in dry air. This secretion therefore seems to bear a relation to transpiration and hence to turgor. As the degree of turgor varies indirectly with the amount of transpiration, other factors being equal, secretion would be least when transpiration is greatest. Turgor, too, is a necessary accompaniment of growth; flaccid tissues do not grow larger. If those influences which affect *R. vernicifera* have a similar action on *R. diversiloba*, then secretion, and consequently the plant conditions for poisoning, would be greatest during that time of the year when the growth of the plant is most active and the tissues least resistant, namely, in the spring. Obviously enough, when the plant is in full leaf and when growth has diminished, its resistance to injury will be greater and the liability of poisoning by it less.

The malignancy of the plant may also be considered in relation to its visibility or conspicuity. From this standpoint the virulency of the plant would be indirectly proportional to its conspicuity. The plant is least conspicuous when it is not in leaf, more conspicuous in the spring when the leaves and flowers are expanding, still more easily recognized when in full leaf, and most likely to be observed when its leaves have assumed their bright autumnal colors.

The virulency of the plant may be summarized according to its toxic portions, the virulency of the resinous sap, the turgescence and ease of fracture of its parts, the conditions of light and atmospheric humidity, and its conspicuity. The liability of poisoning, then, by *R. diversiloba* tissues decreases as follows: immature leaves and flower parts (except anthers and pollen), mature leaves, green stems, young roots, woody stems, and woody roots. According to the amount of poison in the plant, however, virulency would be greatest during the period of full leaf. This factor gives way before the far greater balance of factors just mentioned.

This theoretical consideration of the liability to Rhus poisoning from a botanical point of view has its counterpart in clinical statistics. The latter lend analogous evidence to the conclusion that spring has the greatest number of cases, that a sudden decrease in cases occurs during the time of the autumn tints, and the least number of cases takes place during the dormancy of the plant from November until February. It should be noted, also, that in 1915 the greatest number of cases among Berkeley students (table 1) was in March, previous to the opening of flowers about April 4, and that in 1916 the maximum number of cases occurred during February previous to the maximum flowering period (March 22 to May 1) of that year. This evidence contradicts the belief prevalent among many people that the plant is most malignant during its flowering period. Some of the opinions expressed in medical literature in regard to malignancy are as follows: most cases usually in spring (Busey, 9); most noxious at the period of efflorescence (Yandell, 63); most cases in summer and autumn (Park, 46); greatest activity during the flowering season, from May to October (Philadelphia; Blackwood, 5); most virulent July 1 to September 1 (Hubbard, 29); worst in December when buds are coming out and in May when leaves fall (California; Baldwin, 2); poisonous at all seasons of the year (Philadelphia; Beringer, 3); most poisonous when in bloom (Davis, 1897); cases most prevalent at the season of the year when the foliage is beginning to show itself (Cantrell, 10); most poisonous when in bloom (Harriman, 1898); especially virulent just as the buds come in the spring (Thudichum, 56); more active (in New York) during the summer months, the last two months of spring, and the two first months of autumn (Hadden, 22).

TABLE I. *Occurrence of Rhus diversiloba Dermatitis among University Students, Berkeley, California*

Year	Time and Frequency of Occurrence															
	June (Part of Month)		July		August (Part of Month)			September					October			
	Number		Number		Number		Percent	Number			Percent		Number		Percent	
1912–13 . . .					2		1.5		13		10.4		6		4.8	
					0		2.0	1.5	11	24	9.4	9.9	4	10	3.4	4.1
1913–14 . . .									14		8.9		8		5.0	
									21	35	13.2	11.0	13	21	8.1	6.5
1914–15 . . .					4		2.2		15		8.4		25		14.1	
					3	7	1.6	1.4	16	31	8.8	8.6	22	47	12.2	13.1
1915–16 . . .					9		5.4		25		15.2		18		10.9	
					4	13	3.5	4.4	15	40	13.3	14.2	9	27	8.0	9.4
1916–17 . . .					2		0.8		27		11.5		28		12.4	
					4	6	2.1	1.4	16	43	8.7	10.1	19	47	10.4	11.4
1917–18 . . .	4		4		3		3.8		8		10.2		6		7.6	
	1	5	16	20	3	6	1.9	2.8	18	26	11.6	10.9	13	19	8.3	7.8
1918–19 . . .	0		0		0				0				0			
	6	6	11	11	1	1			0	0			8	8		

TABLE I. (Continued)

Year	November				December (Part of Month)				January (Part of Month)				February				March			
	Number		Percent		Number		Percent		Number		Percent		Number		Percent		Number		Percent	
1912–13.	13		10.4		11		8.8		9		7.2		24		19.3		19		16.1	
	9	22	7.6	9	11	22	9.4	9.1	3	12	2.5	4.8	21	45	17.9	18.6	42	61	35.8	25.9
1913–14.	11		7.0		14		8.9		8		5.0		22		14.0		61		38.8	
	23	34	14.4	10.7	20	34	12.8	10.8	5	13	3.2	4.1	19	41	12.1	13.0	49	110	31.4	35.1
1914–15.	20		11.2		9		5.0		12		6.7		18		10.1		30		16.9	
	30	50	16.6	13.9	6	15	3.3	4.1	10	22	5.5	6.1	21	39	11.6	10.8	43	73	23.8	20.3
1915–16.	19		11.5		10		7.3		6		3.6		32		19.5		45		27.4	
	14	33	12.5	12.0	4	14	3.5	5.4	3	9	2.6	3.1	40	72	35.7	27.5	23	68	20.5	23.9
1916–17.	26		11.1		12		5.1		8		3.4		34		14.5		61		26.1	
	26	52	14.2	12.6	10	22	5.4	5.2	7	15	3.8	3.6	25	59	13.7	14.1	29	90	15.9	21.0
1917–18.	13		16.6		8		10.2		7		8.9		8		10.2		12		15.3	
	24	37	15.4	16.0	9	17	5.8	8.0	15	22	9.6	9.7	19	27	12.2	11.2	29	41	18.7	17.0
1918–19.	2																			
	11	13																		

TABLE I. (Continued)

Year	April				May (Part of Month)				Annual Summaries							
									Total Annual				Total Students in Attendance at Berkeley†		Percentage of Students Affected by *Rhus diversiloba* Treated at University Infirmary	
	Number		Percent		Number		Percent		Number		Percent					
1912–13	18		14.5		9		7.2		124		99.4		2821		4.03	
	11	29	9.4	11.9	5	14	4.2	5.7	117	241	96.6	100.5	1846	4667	6.33	5.18
1913–14	19		12.1		0		0		157		99.7		3285		4.77	
	6	25	3.8	7.9	0		0		156	313	99.0	99.1	2064	5349	7.55	6.16
1914–15	35		19.7		9		5.0		177		99.3		3454		5.12	
	27	62	15.0	17.3	2	11	1.1	3.0	180	357	99.5	98.6	2394	5848	7.52	6.32
1915–16	No record				No record				164		100.8		3491		4.69	
									112	276	99.6	99.9	2706	6197	4.13	4.41
1916–17	28		12.0		7		3.0		233*		99.9		3751		6.21	
	37	65	20.3	16.1	9	16	4.9	3.9	182*	415*	99.4	99.4	2944	6695	6.18	6.19
1917–18	13		16.6		0		0		78		99.4		2765			
	23	36	14.8	15.7	2	2	1.2	0.6	155	233	99.5	99.8	3248	6013	4.7	
1918–19																

Note. The upper figures on the left of each group refer to men, the lower to women; the figures on the right side are the totals.

* Summer school excluded in order that a more true comparison may be made.

† Figures from "Annual report of the President of the University, 1918–19."

1915–16 annual percentages would probably be greater and monthly percentages less if record were complete.

The number of cases of dermatitis from *R. diversiloba* is influenced, not only by the condition of the plant, but also by those conditions which tend to make individuals come in contact with it or with substances coated with its poisonous sap. The clinical statistics, therefore, do not constitute a true index of the virulency of the plant, since the total number of persons exposed is not known. The number of exposed persons in all probability varies at different times of the year, according to the weather conditions, state of other vegetation, individual freedom, etc.

Many attractive wild flowers are found in the same locality with *R. diversiloba* shrubs, such as Clarkias, Godetias, Collinsias, Brodiaeas, and larkspurs (Parsons, 47). John Muir (43) "oftentimes found a curious twining lily (*Stropholieion Californicum*) climbing its branches." The desire to gather spring wild flowers is often greater than the fear of *Rhus diversiloba*. Circumstances thus combine to bring a person in contact with the plant at the time when it is capable of doing the most harm.

LITERATURE CITED

1. **Abrams, LeRoy.** Flora of Los Angeles and vicinity. 1911.
2. **Baldwin, A. E.** A case of poisoning by *Rhus Toxicondendron.* Pacific Medical and Surgical Journal **30**: 509, 643. 1887.
3. **Beringer, G. M.** Rhus poisoning. Amer. Jour. Pharm. **68**: 18–20. 1896.
4. **Bigelow, J.** American medical botany **3**: 20. Boston, 1820.
5. **Blackwood, W. R. D.** Some thoughts on Rhus poisoning. Abst. New Rem. 9: 278. 1886. Abst. New Rem. **10**: 8. 1881. Abst. Amer. Philos. Assoc. Proc. **29**: 230. 1881. Philadelphia Med. Times **10**: 618. 1880.
6. **Brandegee, T. S.** A collection of plants from Baja California. Proc. Calif. Academy of Sciences, ser. 2, **2**: 140. 1889.
7. ———. The plants of Santa Catalina Island. Zoe **1**: 110, 134. 1890.
8. **Brewer, W. H., Watson, S., and Gray, A.** Botany (of California), ed. 2, **1**: 110. Cambridge, 1876.
9. **Busey, S. C.** Poisoning by the *Rhus Toxicodendron.* Amer. Jour. Med. Sci. **17**: 436–442. 1873.
10. **Cantrell, J. A.** Relative value of certain drugs in the treatment of ivy poisoning. New Eng. Med. Monthly **17**: 270–272. 1898.
11. ———. The Philadelphia Polyclinic **7**: 20. 1898.
12. **Chestnut, V. K.** Plants used by Indians of Mendocino County, California. Contrib. U. S. Nat. Herb. **7**: 364. 1902.
13. **Coville, F. V.** Botany of Death Valley expedition. Contrib. U. S. Nat. Herb. **4**: 81. 1893.
14. **Elliott, S.** Cited by Bigelow, 4.
15. **Engler, A., and Prantl, K.** Die natürlichen Pflanzenfamilien III, **5**: 168. 1897.
16. **Gray, A.** Botany. In "Geological Survey of California" **1**: 110. 1826.
17. ———. Synoptical flora **1**, fasc. 12: 382–383. 1895–1897.
18. **Gray, A., Watson, S., and Robinson, B. L.** Synoptical flora of North America, ed. 2, 1^1: 383. New York, 1897.
19. ———. Studies in the botany of California and parts adjacent. Bull. Cal. Acad. Sci. **2**: 393. 1886–1887.
20. **Greene, E. L.** Manual of botany, p. 73. San Francisco, 1894.
21. ———. Leaflets of botanical observation and criticism **1**: 119. Washington, D. C., 1903–1906.

22. **Hadden, A.** Poison ivy or *Rhus Toxicodendron.* Med. Review of Reviews **12**: 764. 1906.
23. **Hall, H. M.** A botanical survey of San Jacinto Mountains. Univ. Cal. Pub. Bot. **1**: 1–140. 1902.
24. ————. A Yosemite flora, p. 151. 1912.
25. **Hooker, J. D., and Jackson, B. D.** Index Kewensis. Part IV, p. 714. Oxford, 1895.
26. **Hooker, W. J.** Flora boreali americana **1**: 126, 127. London, 1831.
27. **Hooker, W. J., and Arnott, J. A. W.** The botany of Captain Beechey's voyage, part 3, p. 137. London, 1832.
28. **Howell, T.** A flora of northwest America, p. 119. Portland, Ore., 1898. Ditto, vol. 1, p. 119. Portland, Ore., 1903.
29. **Hubbard, S.** *Rhus Toxicodendron.* Peoria Med. Monthly **6**: 323. 1885–1886.
30. **Inui, T.** Ueber den Gummiharz-Gang des Lackbaumes und seiner verwandten Arten (Abstr.). Bot. Centralbl. **83**: 352. 1900.
31. **Jepson, W. L.** A school flora for the Pacific Coast, p. 38. New York, 1902.
32. ————. A flora of western and middle California, p. 250. Berkeley, 1901. 2d edition, p. 249. Berkeley, 1911.
33. **Kalm, P.** Travels into North America (Eng. transl.), ed. 2, **1**: 53, 60–64, 139; **2**: 20. London, 1772.
34. ————. Travels into North America, etc. (Eng. transl. by John Reinhold Forster). In Pinkerton, J. "A general collection of the best and most interesting voyages and travels" **13**: 402–403, 434. 1812.
35. **Kunze, R. E.** Poison Rhus. Med. Tribune **5**: 111–120. 1883.
36. **Lindley, J.** *Rhus diversiloba.* Various leaved poison oak. Edwards' Bot. Reg. n. ser. **18**: 38. 1845.
37. **Linnaeus, C.** Hortus cliffortianus, p. 110. Amsterdam, 1737.
38. ————. Species plantarum, pp. 265, 266. Stockholm, 1778.
39. **Lyon, W. S.** The flora of our southwestern Archipelago II. Bot. Gaz. **2**: 333. 1886.
40. **McNair, J. B.** The oxidase of *Rhus diversiloba.* Jour. Infect. Dis. **20**: 485–498. 1917.
41. **Michaux, A.** Cited by Bigelow, 4.
42. **Möbius, M. A.** Der Japanische Lackbaum, *Rhus vernicifera* D. C. Abhandl. Senckenberg. Naturforsch. Ges. **20**: 201–247. Frankfort, 1899.
43. **Muir, J.** My first summer in the Sierra, pp. 34, 35. New York, 1911.
44. **Newberry, J. S.** Botanical report. In U. S. War Dept., Reports of explorations . . . from the Mississippi river to the Pacific ocean, 1854–1855. **6**: 69. Washington, 1857.
45. **Nuttall, T.** Cited by Bigelow, 4.
46. **Park, R.** Dermatitis venenata; or, *Rhus Toxicodendron* and its action. Archives of Dermatology **5**: 227–234. 1879.
47. **Parsons, Mary E.** The wild flowers of California; their names, haunts and habits, p. 8 (corrected edition). San Francisco, 1907.
48. **Piper, C. V.** Flora of the state of Washington. Contrib. U. S. Nat. Herb. **9**: 384. 1906.
49. **Pursh, F. T.** Cited by Bigelow, 4.
50. **Rost, E., and Gilg, E.** Der Giftsumach, *Rhus Toxicodendron* L., und seine Giftwirkungen. Ber. Deutsch. Pharm. Ges. **22**: 296–358. 1912.
51. **Schwalbe, C.** On the active principle of *Rhus diversiloba* (poison oak). Med. Record **63**: 855. 1903.
52. **Schwalbe, K.** Die giftigen Arten der Familie Rhus: *R. diversiloba, R. Toxicodendron* und *R. venenata.* Münchener Medic. Wochenschr. **49**: 1616. 1902.
53. **Smith, J.** The general history of the Bermudas, now called the Summer Isles; from their beginning in the year of our Lord 1593, to this present 1624, etc. In Book V of "General History of Virginia, New England and Summer Isles," by Captain John Smith. In Pinkerton, J. "A general collection of the best and most interesting voyages and travels" **13**: 172. 1812.

54. ————. (1609) The Historye of the Bermudaes or Summer Islands, p. 2. Edited by J. H. Lefroy. Publ. by the Hakluyt Society. London, 1882.
55. **Stirling, E. C.** An eruption of the skin caused by the poison ivy. Australas. Med. Gaz. **33**: 355–359. 1913.
56. **Thudichum, C. L.** The alkaloidal clinic **10**: 829. 1903.
57. **Torrey, J.** Pacific Railroad report. Route near 35th parallel explored by Whipple in 1853–54. No. 4, Report of the botany of the expedition. 1856.
58. **Torrey, J., and Gray, A.** Flora of North America **1**: 217–218, 681. New York and London, 1839 and 1843.
59. **Warren, L. E.** Some observations on the pollen of poison sumach. Amer. Jour. Pharm. **85**: 545–549. 1913.
60. **Wheeler, G. M.** Report of the United States Geological Survey west of the 100th meridian, p. 42. Washington, 1878.
61. **White, J. C.** On the action of *Rhus venenata* and *Rhus Toxicodendron.* New Yor Med. Jour. **17**: 225–249. 1873.
62. ————. Dermatitis venenata. Boston, 1887.
63. **Yandell, L. P.** Poison oak eruption. Louisville Med. News **2**: 32. 1876.
64. **Young, A. H.** *Monotropa uniflora.* Bot. Gaz. **3**: 37, 38. 1878.

DESCRIPTION OF PLATE II

The material selected for the sections was fixed in a chrom-acetic fixative (1 percent by weight chromic acid in water, 0.5 percent glacial acetic acid by weight). Suitable pieces were then hardened by placing in alcohol of different concentrations in series of 6 percent, 12 percent, 25 percent, 50 percent, and 75 percent, then in xylol and paraffine and finally in paraffine. Sections were then cut on the microtome in series, fastened to clean slides with egg albumen, stained with safranin and Delafield's haemotoxylin, and finally washed in absolute alcohol, in xylol, and mounted in balsam. By this treatment lignified and suberized walls were stained red and cellulose walls violet.

The photomicrographs were made with an electric arc as the source of light. The amount of magnification was calculated by the aid of a ruled slide on the microscope stage and of a rule on the ground glass focusing screen, using the same lenses as were used in the exposure. The photomicrographs have been reduced 2⅔ times.

A. Transverse section through a lateral leaf rib, showing a resin duct. The resin duct is 0.0053 mm. in diameter. The shortest distance to the bast ring which surrounds the resin duct is 0.0056 mm. × 168.4.

B. Young shoots showing simultaneous expansion of leaves and flower panicles (reduced one fifth).

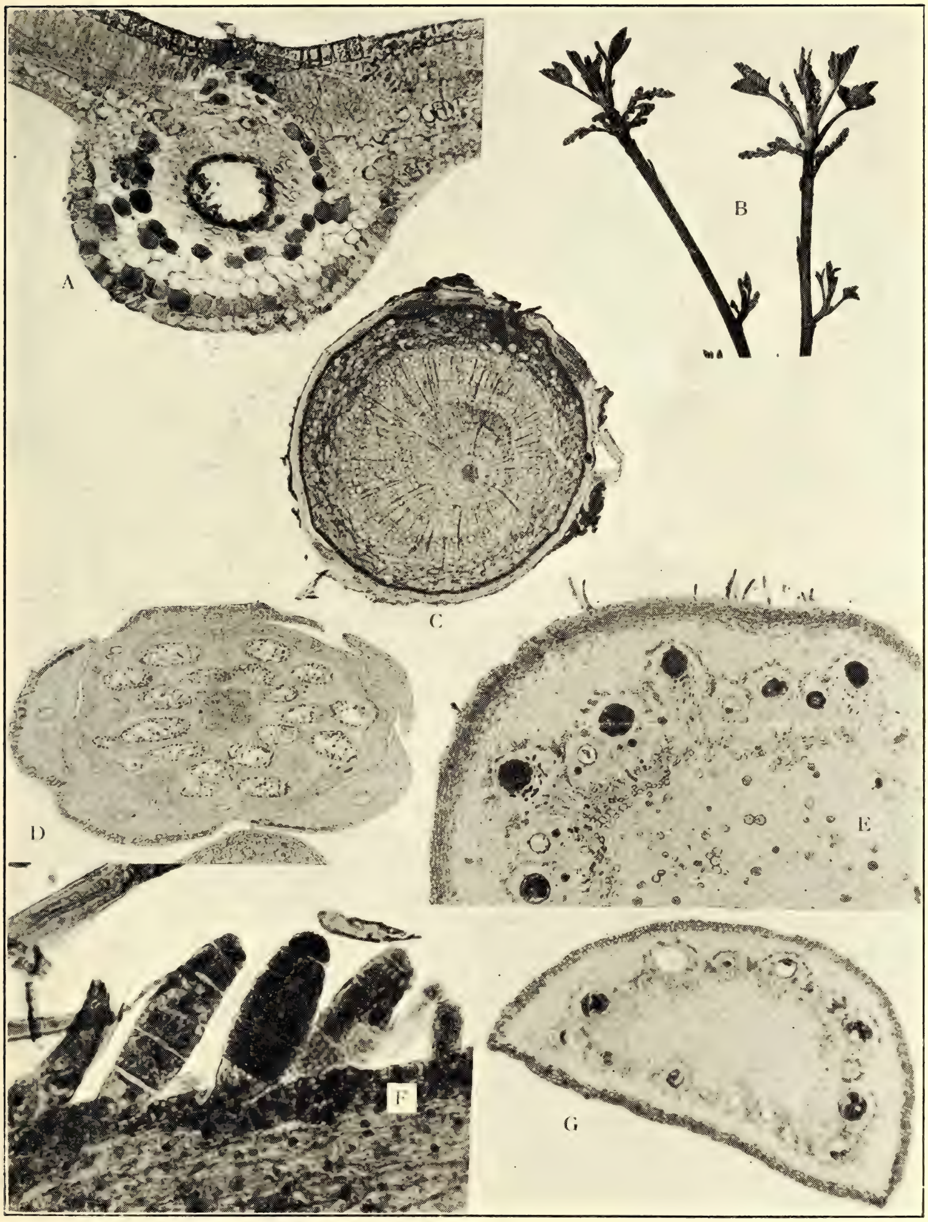
C. Transverse section through a woody root. × 7.5.

D. Transverse section through a staminate flower near the apex showing five calyx leaves with resin ducts, five petals with resin ducts, five anthers showing absence of resin ducts and presence of pollen, and the non-fertile ovule. × 40.9.

E. Transverse section through a green stem, showing epidermis with its trichomes, collenchyma, cortical parenchyma, pericycle with sclerenchyma cells or bast fibers, and thin-walled pericycle parenchyma. × 40.9.

F. Leaf epidermis with attached club-shaped trichomes. (Size of trichomes 0.071 × 0.0027 mm.) × 353.7.

G. Transverse sections of petiole. The largest resin duct has a diameter of 0.01 mm.; the smallest is 0.0044 mm. in diameter. The largest pith cells are larger than the smallest resin ducts. It is 0.02 mm. from the corner of the petiole section to the bast ring which surrounds the nearest resin duct. × 33.8.



McNAIR: TOXICITY OF RHUS DIVERSILOBA.

THE EFFECT OF SALT PROPORTIONS AND CONCENTRATION ON THE GROWTH OF ASPERGILLUS NIGER[1]

C. M. Haenseler

(Received for publication September 21, 1920)

Introduction

A great deal of work has been done in recent years on the various problems of plant nutrition, and especially on the physiological balance of nutrient solutions and on the salt requirements of plants. Although these questions have received some attention ever since the introduction of water cultures by Sachs and Knop, they had never been carried to such logical completeness as was done by Tottingham (9) in his work on the study of the effect upon plant growth of varying the total concentration and salt proportions in Knop's solution. In 1915 Shive (6), using Tottingham's systematic methods, made similar exhaustive studies of a nutrient solution containing only three salts and a trace of iron. This three-salt solution, when properly balanced, gave a better yield than Tottingham's best four-salt solution and has the further advantage of being the simplest satisfactory water culture that had been used up to that time.

Since the introduction of Shive's three-salt solution, which, on account of its relative simplicity, is especially suitable for studies in plant nutrition, it has been used extensively by Shive (6), McCall (4), Hibbard (3), and others for various physiological studies with green plants.

Fundamental problems of nutrition have also been studied extensively in certain fungi and especially in *Aspergillus niger*. The greater number of these studies have dealt with carbon or nitrogen assimilation and with the toxic or stimulative action of various substances. The rôle played by total salt concentration and by the salt proportions of the nutrient medium has apparently received very little attention. There has been no systematic study made upon any fungus which corresponds to the nutrition studies made with green plants by Tottingham, Shive, and others. The results of these authors have proven of such fundamental importance in nutrition studies in higher plants that it was thought advisable to test the adaptability of their methods in similar studies upon fungi.

In order to make this test, a series of experiments were planned to study the effect upon *Aspergillus niger* of varying the total salt concentration and salt proportions in a simple nutrient solution.

---

[1] Paper No. 21 of the Technical Series, New Jersey Agricultural Experiment Stations, Department of Plant Physiology.

The methods used by Shive (6) in his studies on the physiological balance of nutrient solutions for higher plants were used as a basis for this work. A number of radical modifications in these methods, however, were necessary on account of the physiological differences between the green plants and fungi. The most essential modification was in the composition of the nutrient solution itself. In Shive's solution three mineral salts and a trace of iron constitute the nutrient material, and for green plants these salts contain all the necessary elements for growth. For fungi, however, a source of energy must be supplied in addition to the mineral salts, and in this work it was supplied in the form of cane sugar. A second necessary modification was in the treatment of the solution. In water-culture work with higher plants it is neither essential nor practicable always to use sterile solutions. With the fungi, on the other hand, it is essential that the medium be sterilized thoroughly before inoculating with the desired organism. The sterilization process as used for these experiments, however, probably caused very little alteration of the medium and may be overlooked as a factor influencing the medium itself.

This work was outlined primarily to see whether the methods which Shive and others have used with such marked success in nutrition studies with green plants, would prove equally useful for similar studies with fungi.

This work was carried out under the direction of Dr. J. W. Shive in the Laboratory of Plant Physiology at the New Jersey Agricultural Experiment Station.

Experimental Methods

The experiments herein discussed consist of two groups of cultures. Group 1, comprising series 1, 2, and 3, contains $Ca(NO_3)_2$ as the source of nitrogen and will be referred to as the $Ca(NO_3)_2$ group. Group 2, comprising series 4 and 5, contains $NaNO_3$ as the source of nitrogen and will be designated the $NaNO_3$ group.

Aspergillus niger was grown in 250 cc. Jena glass Erlenmeyer flasks on 50 cc. of a liquid medium containing three nutrient salts, cane sugar, and a trace of iron. Each culture throughout five series contained the same amount of iron and sugar, the iron being present in amounts equivalent to 0.01 gram of ferrous sulphate per liter, and the sugar in amounts equivalent to 38.97 grams per liter of nutrient solution.

In series 1, the nutrient salts, KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$, are present in quantities sufficient to give a total calculated osmotic concentration value of 0.5 atmospheres. The series consists of 36 cultures, each differing from all the other cultures of the series in the proportions of the three nutrient salts. The 36 cultures represent all the possible proportions or combinations obtainable by varying the partial concentration of each of the salts by increments of one tenth of the total concentration. A full account of this method of studying the effects of salt proportions is given by Shive (6) and need not be further discussed here.

Series 2 and 3 differ from series 1 only in total salt concentration, the total calculated osmotic concentration values in these series being 2.1 and 4.2 atmospheres respectively. Series 4 and 5 have the same total concentration values as series 2 and 3 respectively, but in the former $NaNO_3$ is used instead of $Ca(NO_3)_2$. For purposes of comparison the standard nutrient solution proposed by Thom (8) was here used as a check in each series (except series 4), always with the same total osmotic salt concentration as that employed for the series in which it occurred. In Tables 1 and 2 are given the actual partial volume-molecular concentrations of each of the solutions of the five series used in this study. The culture numbers refer to the positions which the cultures occupy on the triangular diagram[2] graphically representing the series with respect to the osmotic proportions of the three salts employed.

TABLE 1. *Partial volume-molecular concentration of the salts employed in the culture solutions of the series in group 1*

Culture No.	Series 1 (0.5 Atm.)			Series 2 (2.1 Atm.)			Series 3 (4.2 Atm.)		
	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$
R1C1	.00111	.00070	.00941	.00444	.00302	.04157	.00888	.00625	.08648
R1C2	.00111	.00141	.00824	.00444	.00604	.03637	.00888	.01250	.07567
R1C3	.00111	.00211	.00706	.00444	.00906	.03118	.00888	.01875	.06486
R1C4	.00111	.00282	.00588	.00444	.01208	.02598	.00888	.02500	.05405
R1C5	.00111	.00352	.00471	.00444	.01510	.02078	.00888	.03125	.04234
R1C6	.00111	.00423	.00353	.00444	.01811	.01559	.00888	.03749	.03243
R1C7	.00111	.00493	.00235	.00444	.02113	.01039	.00888	.04374	.02162
R1C8	.00111	.00563	.00118	.00444	.02415	.00520	.00888	.04999	.01081
R2C1	.00222	.00070	.00824	.00888	.00302	.03637	.01776	.00625	.07567
R2C2	.00222	.00141	.00706	.00888	.00604	.03118	.01776	.01250	.06486
R2C3	.00222	.00211	.00588	.00888	.00906	.02598	.01776	.01875	.05405
R2C4	.00222	.00282	.00471	.00888	.01208	.02078	.01776	.02500	.04324
R2C5	.00222	.00352	.00353	.00888	.01510	.01559	.01776	.03125	.03243
R2C6	.00222	.00423	.00235	.00888	.01811	.01039	.01776	.03749	.02162
R2C7	.00222	.00493	.00118	.00888	.02113	.00520	.01776	.04374	.01081
R3C1	.00333	.00070	.00706	.01332	.00302	.03118	.02664	.00625	.06486
R3C2	.00333	.00141	.00588	.01332	.00604	.02598	.02664	.01250	.05405
R3C3	.00333	.00211	.00471	.01332	.00906	.02078	.02664	.01875	.04324
R3C4	.00333	.00282	.00353	.01332	.01208	.01559	.02664	.02500	.03243
R3C5	.00333	.00352	.00235	.01332	.01510	.01039	.02664	.03125	.02162
R3C6	.00333	.00423	.00118	.01332	.01811	.00520	.02664	.03749	.01081
R4C1	.00444	.00070	.00588	.01776	.00302	.02598	.03552	.00625	.05405
R4C2	.00444	.00141	.00471	.01776	.00604	.02078	.03552	.01250	.04324
R4C3	.00444	.00211	.00353	.01776	.00906	.01559	.03552	.01875	.03243
R4C4	.00444	.00282	.00235	.01776	.01208	.01039	.03552	.02500	.02162
R4C5	.00444	.00352	.00118	.01776	.01510	.00520	.03552	.03125	.01081
R5C1	.00555	.00070	.00471	.02220	.00302	.02078	.04440	.00625	.04324
R5C2	.00555	.00141	.00353	.02220	.00604	.01559	.04440	.01250	.03243
R5C3	.00555	.00211	.00235	.02220	.00906	.01039	.04440	.01875	.02162
R5C4	.00555	.00282	.00118	.02220	.01208	.00520	.04440	.02500	.01081
R6C1	.00666	.00070	.00353	.02664	.00302	.01559	.05328	.00625	.03243
R6C2	.00666	.00141	.00235	.02664	.00604	.01039	.05328	.01250	.02162
R6C3	.00666	.00211	.00118	.02664	.00906	.00520	.05328	.01875	.01081
R7C1	.00777	.00070	.00235	.03108	.00302	.01039	.06216	.00625	.02162
R7C2	.00777	.00141	.00118	.03108	.00604	.00520	.06216	.01250	.01081
R8C1	.00888	.00070	.00118	.03552	.00302	.00520	.07104	.00625	.01081

---

[2] For a description of this triangular diagrammatic scheme see Shive (6), McCall (4), Hibbard (3).

TABLE 2. *Partial volume-molecular concentration of the salts employed in the cultures of the series in group* 2

Culture No.	Series 4 (2.1 Atm.)			Series 5 (4.2 Atm.)		
	KH_2PO_4	$NaNO_3$	$MgSO_4$	KH_2PO_4	$NaNO_3$	$MgSO_4$
R1C1	.00444	.00414	.04157	.00888	.00860	.08648
R1C2	.00444	.00829	.03637	.00888	.01721	.07567
R1C3	.00444	.01243	.03118	.00888	.02581	.06486
R1C4	.00444	.01658	.02598	.00888	.03442	.05405
R1C5	.00444	.02072	.02078	.00888	.04302	.04324
R1C6	.00444	.02487	.01559	.00888	.05162	.03243
R1C7	.00444	.02901	.01039	.00888	.06023	.02162
R1C8	.00444	.03316	.00502	.00888	.06883	.01081
R2C1	.00888	.00414	.03637	.01776	.00860	.07567
R2C2	.00888	.00829	.03118	.01776	.01721	.06486
R2C3	.00888	.01243	.02598	.01776	.02581	.05405
R2C4	.00888	.01658	.02078	.01776	.03442	.04324
R2C5	.00888	.02072	.01559	.01776	.04302	.03243
R2C6	.00888	.02487	.01039	.01776	.05162	.02162
R2C7	.00888	.02901	.00502	.01776	.06023	.01081
R3C1	.01332	.00414	.03118	.02664	.00860	.06486
R3C2	.01332	.00829	.02598	.02664	.01721	.05405
R3C3	.01332	.01243	.02078	.02664	.02581	.04324
R3C4	.01332	.01658	.01559	.02664	.03442	.03243
R3C5	.01332	.02072	.01039	.02664	.04302	.02162
R3C6	.01332	.02487	.00502	.02664	.05162	.01081
R4C1	.01776	.00414	.02598	.03552	.00860	.05405
R4C2	.01776	.00829	.02078	.03552	.01721	.04324
R4C3	.01776	.01243	.01559	.03552	.02581	.03243
R4C4	.01776	.01658	.01039	.03552	.03442	.02162
R4C5	.01776	.02072	.00502	.03552	.04302	.01081
R5C1	.02220	.00414	.02078	.04440	.00860	.04324
R5C2	.02220	.00829	.01559	.04440	.01721	.03243
R5C3	.02220	.01243	.01039	.04440	.02581	.02162
R5C4	.02220	.01658	.00502	.04440	.03442	.01081
R6C1	.02664	.00414	.01559	.05328	.00860	.03243
R6C2	.02664	.00829	.01039	.05328	.01721	.02162
R6C3	.02664	.01243	.00502	.05328	.02581	.01081
R7C1	.03108	.00414	.01039	.06216	.00860	.02162
R7C2	.03108	.00829	.00502	.06216	.01721	.01081
R8C1	.03552	.00414	.00502	.07104	.00860	.01081

For each series a stock solution of each of the salts was made up with distilled water to such a concentration that 1 cc. of the stock in 50 cc. of the culture solution produced in any culture one tenth of its total required salt concentration. Thus, the culture in which the three salts KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$ produced, respectively, 1/10, 6/10, and 3/10 of the total concentration, received of these stocks 1 cc., 6 cc., and 3 cc., in the order given. Each culture, therefore, received a total of 10 cc. of stock solutions, leaving 40 cc. to be supplied by other means. The sugar stock was made up to 5/4 the concentration desired in the finished culture; thus, 40 cc. of this stock contained the proper amount of sugar for the 50 cc. culture. Ferrous sulphate equivalent to 0.01 gram $FeSO_4$ per liter of finished nutrient was added directly to the sugar stock.

The stock solutions were weighed and sterilized separately at 100° C. for one hour on three consecutive days. The loss by evaporation was replaced by sterile distilled water. After cooling, the solutions were forced by air pressure through glass tubes into burettes and the proper amount of each was carefully measured into the culture flask. All glassware and stoppers were sterilized either in an autoclave or in a hot-air chamber immediately before using. In order to reduce the chances of outside contamination, the cultures were made up and inoculated in a dust-proof inoculation chamber. A number of uninoculated check cultures proved that they had been made under perfectly sterile conditions.

It would have been more convenient to make up the cultures before sterilization, but this method was not considered advisable on account of the decomposition of the salts which occurs while heating a mixed salt solution. Some of the solutions employed, especially those with a relatively large amount of KH_2PO_4, were quite unstable at the boiling point, and even at 30° C. a few of the more concentrated cultures contained a slight precipitate. The stock solutions were, therefore, sterilized separately and the culture solutions were prepared from these under aseptic conditions.

Baker's analyzed salts were used throughout. On account of the uncertainty as to the exact amount of water of crystallization in the calcium nitrate, this salt was freed from its water of crystallization by carefully fusing and dehydrating at a final temperature of 150° C. A fine grade of commercial granulated sugar was used.

The cultures were well shaken and inoculated heavily with spores from a one-week-old agar culture of *Aspergillus niger*. Enough spores were transferred in the inoculation to produce a very thin, but visible, uniform film on the surface of the culture. This heavy inoculation was found necessary to get a uniform growth. Light inoculations were apt to give "islands" of growth instead of a uniform film over the entire surface. A preliminary test to determine the possible error caused by unequal inoculation showed that the amount of inoculum may be varied considerably without affecting the yield, provided the sowing is uniform. The amount of inoculum used in these experiments could be reduced to one half or doubled without affecting the yield.

The cultures were incubated at 29° to 30° C. for seven days. It has been shown by Brenner (2) and others that *Aspergillus niger* in a good nutrient medium makes its full growth in from three to five days, and that there is a gradual loss in dry weight after that time. A seven-day growing period was chosen in this work in order to give the poorer cultures a chance to get their full development. Under the conditions of these experiments it was found that harvesting could be delayed until the seventh day without affecting the results appreciably, as was shown by an experiment to determine the growth curve in one of the best solutions used in this work (culture R1C8, series 3). In this experiment fourteen cultures were inoculated and two were harvested each day for seven consecutive days. The graph in figure 1, which is self explanatory, shows the result of this experiment. From this graph it will be seen that although the maximum growth was reached at the end of the third day, there was only a very slight decline up to the end of the seventh day. No appreciable loss is thus sustained by allowing the growth to proceed for seven days.

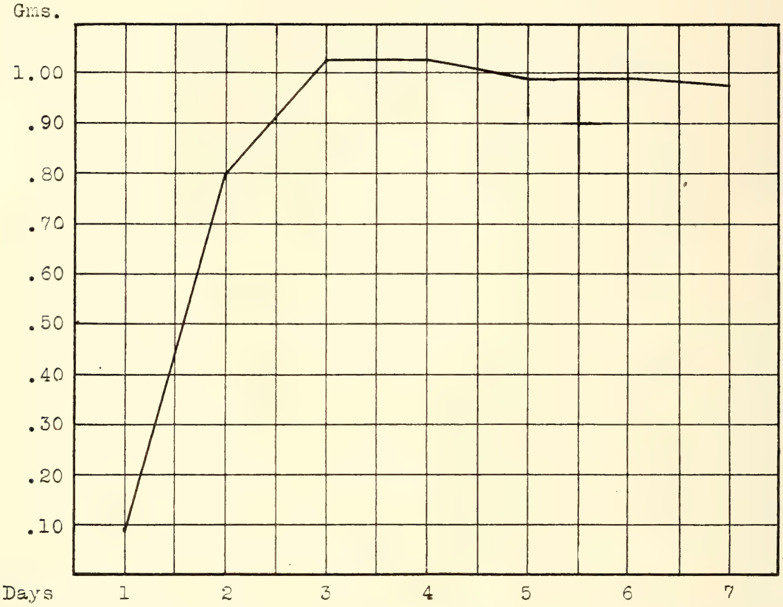


Fig. 1. Graph showing the rate of growth of *Aspergillus niger* in a three-salt medium.

At the end of the growing period the fungus was placed upon a dried and weighed filter paper, washed with water, placed with filter paper into weighing bottles, dried to constant weight at a final temperature of 104° C., and weighed to the nearest milligram.

Effect of Increasing Total Salt Concentration on the Growth of Aspergillus niger

Table 3 gives the absolute dry weights of the fungus from the cultures in each of the five series. By reading the yields across the columns in this table, it is seen that the dry weights increase as the total salt concentration increases. This increase is shown for all corresponding cultures, regardless of the salt proportions. Figure 2, in which yields are plotted in the form of graphs, shows the relation between total concentration and yield more clearly than does table 3. In these curves the dry-weight yields of series 2 have been plotted in the descending order of their values and the yields from the other series are plotted with the same culture order as series 2. The light lines represent the yields from series 1, 2, and 3, which differ from each other, with respect to the composition of the solutions used, only in

TABLE 3. *Dry weights of fungus obtained from the cultures of series 1 to 5, and the yield ratios between cultures having the same salt proportions but varying in total concentration*

Culture No.	Ca(NO_3)$_2$ Group					NaNO$_3$ Group		
	Dry Weight of Fungus			Ratio		Dry Weight of Fungus		Ratio
	Ser. 1 (0.5 Atm.)	Ser. 2 (2.1 Atm.)	Ser. 3 (4.2 Atm.)	$\frac{\text{Ser. 2}}{\text{Ser. 1}}$	$\frac{\text{Ser. 3}}{\text{Ser. 2}}$	Ser. 4 (2.1 Atm.)	Ser. 5 (4.2 Atm.)	$\frac{\text{Ser. 5}}{\text{Ser. 4}}$
R1C1...	.065	.173	.347	2.66	2.01	.098	.199	2.03
R1C2...	.114	.343	.624	3.01	1.82	.194	.382	1.97
R1C3...	.168	.474	.874	2.82	1.84	.292	.553	1.89
R1C4...	.209	.606	.956	2.90	**1.58**	.369	.709	1.92
R1C5...	.248	.762	.949	3.07	**1.25**	.457	.775	**1.70**
R1C6...	.282	.848	.983	3.01	**1.16**	.544	.754	**1.39**
R1C7...	.302	.900	.985	2.98	**1.09**	.621	.723	**1.16**
R1C8...	.328	.915	.977	2.79	**1.07**	.665	.701	**1.05**
R2C1...	.062	.174	.351	2.81	2.02	.108	.202	1.87
R2C2...	.103	.331	.632	3.21	1.91	.214	.390	1.82
R2C3...	.159	.477	.865	3.00	1.81	.282	.564	2.00
R2C4...	.199	.625	.947	3.14	**1.52**	.369	.721	1.95
R2C5...	.249	.736	.969	2.96	**1.32**	.454	.777	**1.71**
R2C6...	.281	.835	.984	2.97	**1.18**	.546	.743	**1.36**
R2C7...	.307	.900	.991	2.93	**1.10**	.610	.711	**1.17**
R3C1...	.058	.182	.355	3.14	1.95	.102	.197	1.93
R3C2...	.103	.334	.610	3.24	1.83	.193	.386	2.00
R3C3...	.151	.477	.875	3.16	1.83	.283	.560	1.98
R3C4...	.205	.605	.957	2.95	**1.58**	.373	.714	1.92
R3C5...	.243	.730	.957	3.00	**1.31**	.456	.766	**1.68**
R3C6...	.276	.824	.976	2.99	**1.18**	.551	.743	**1.35**
R4C1...	.061	.189	.341	3.10	1.80	.101	.203	2.01
R4C2...	.101	.330	.603	3.27	1.83	.193	.383	1.98
R4C3...	.159	.491	.874	3.09	1.78	.294	.565	1.92
R4C4...	.207	.600	.960	2.90	**1.60**	.369	.731	1.98
R4C5...	.231	.730	.966	3.16	**1.32**	.468	.759	**1.62**
R5C1...	.056	.180	.354	3.21	1.97	.104	.191	1.84
R5C2...	.110	.340	.634	3.09	1.86	.192	.373	1.94
R5C3...	.148	.477	.867	3.22	1.82	.274	.576	2.10
R5C4...	.203	.599	.958	2.95	**1.60**	.366	.730	1.99
R6C1...	.060	.181	.364	3.02	2.01	.106	.194	1.83
R6C2...	.119	.343	636	2.88	1.85	.188	.378	2.01
R6C3...	.148	.479	.886	3.24	1.85	.285	.555	1.95
R7C1...	.055	.186	.352	3.38	1.89	.091	.193	2.12
R7C2...	.101	.326	.625	3.23	1.92	.204	.358	1.76
R8C1...	.059	.194	.324	3.29	1.67	.092	.192	2.09
Check	.201	.647	.733				.730	

total salt concentration. It will be noted that series 1, which has a total osmotic salt-concentration value of 0.5 atmospheres, gave relatively low yields throughout all the salt proportions. In series 2, with a total osmotic salt-concentration value of 2.1 atmospheres, the yields are very much higher, and in series 3, with a concentration double that of series 2, the yields are still higher. Likewise in series 4 and 5 (represented graphically in figure 2 by the heavy lines), markedly higher yields are shown for series 5 than for series 4 in which a much lower total salt concentration was employed.

This direct correlation between total salt concentration and yield is quite clearly brought out in figure 2 by comparing each individual culture in series 2 with its corresponding cultures in the other series. Such a comparison between the yields from series 1 and series 2 indicates that series 2, with a total concentration value four times that of series 1, gave yields throughout which were approximately three times as great as the corresponding yields from series 1. The exact ratios of the yields from series 2 to the corresponding ones from series 1 are given in table 3, as are also similar ratios between the yields from series 2 and 3, and between those from series 4 and 5. A relation similar to the one here pointed out between total salt concentration and yield exists throughout all the series, as is clearly shown by the graphs of figure 2, and by the columns of ratios in table 3. This relation disappears, however, in the high-yielding cultures of series 3. It was found that in these cultures the sugar supply was sufficiently exhausted to become a limiting factor for growth, thus preventing changes in the total salt concentration from showing their true effects. If these high-yielding cultures are omitted from the comparison and only the 21 cultures of series 3 which have low or medium yields are compared with the corresponding cultures of series 2, it will be found that the yields from series 3 are approximately twice as great as are the corresponding yields from series 2, while the total salt concentration in series 3 is double that in series 2. A similar comparison of the corresponding 21 cultures of series 4 and 5 shows a similar correlation between concentration and yield. The ratio of the total salt concentration between series 4 and 5 is 1 : 2, while the ratios between the yields of the corresponding cultures is also approximately 1 : 2, which indicates that by doubling the total osmotic concentration of the solutions without altering the salt proportions, the yields are also approximately

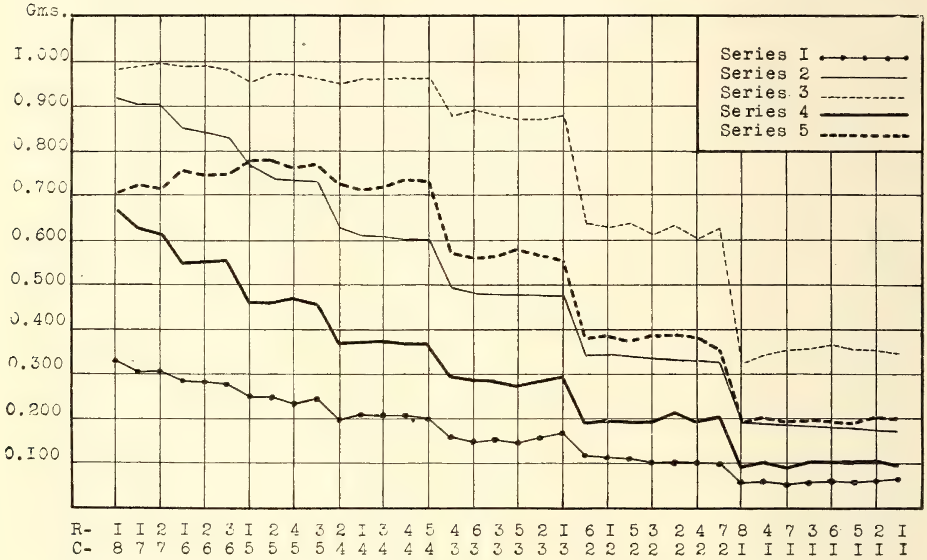


FIG. 2. Graphs showing the absolute yields from the cultures of series 1 to 5.

doubled, as is clearly shown by the ratios in the last column of table 3. The ratios derived from the yield values of the cultures in series 3 and 5 which do not show this relation are indicated in table 3 in bold-face type.

EFFECT OF VARYING THE SALT PROPORTIONS ON THE GROWTH OF ASPERGILLUS NIGER

To facilitate the comparison between the yields with respect to the variations in the salt proportions, the yield values are presented in graphic form in the triangular diagrams of figures 3 and 4, in which each culture occupies a definite position according to the proportions of the salts it contains. In these triangles the individual cultures are numbered according to the row in which they occur (R1, R2, R3, etc.) and according to their position in the row (C1, C2, C3, etc.) According to this triangular diagram (6) the osmotic proportions of the salts in any culture are indicated by its position on the triangle. The partial volume-molecular concentrations corresponding to these proportions are found in table 1 or in table 2. The actual dry weight of fungus derived from each culture is given just opposite the point of the triangle representing that culture. The areas on the triangles including the high, medium, and low yields are separated by dotted lines. The area at the lower right of each triangle includes the cultures giving the highest nine yields, while the cultures giving the lowest nine yields are embraced in the area lying along the left margin of each triangle. The central region on each triangle includes the cultures giving medium yields. In series 5, however, the three cultures at the extreme lower right of the triangle fall into the medium-yield area.

Considering first the effects of changing the partial concentration of KH_2PO_4 and $MgSO_4$, it will be observed in series 1 (fig. 3) that all the C1 cultures (on the left margin of the triangle) gave approximately the same yields. All these cultures contain the same amount of calcium nitrate but differ widely in their proportions of KH_2PO_4 and $MgSO_4$. The partial concentration due to each of these latter two salts varies from 1/10 to 8/10 of the total salt concentration, but in spite of the wide differences in the partial concentrations due to these two salts, all the cultures show approximately equal yield values. This same indifference of the fungus to changes in the partial concentration of KH_2PO_4 and $MgSO_4$ is shown by all cultures in which these two salts are the only variables. The C1 cultures of any one series have very nearly equal yield values. Likewise, all the C2 cultures or C3 cultures, etc., of any particular series gave approximately equal yields. In series 1, where the total concentration is only 0.5 atmosphere, or in series 3, with a total concentration value of 4.2 atmospheres, the fungus is apparently indifferent to the wide variations in the proportions of KH_2PO_4 or of $MgSO_4$. Thus all these series point to the conclusion that within wide limits neither KH_2PO_4 nor $MgSO_4$ tends either to stimulate or to inhibit growth in *Aspergillus niger*. There is no evidence of a physiologically

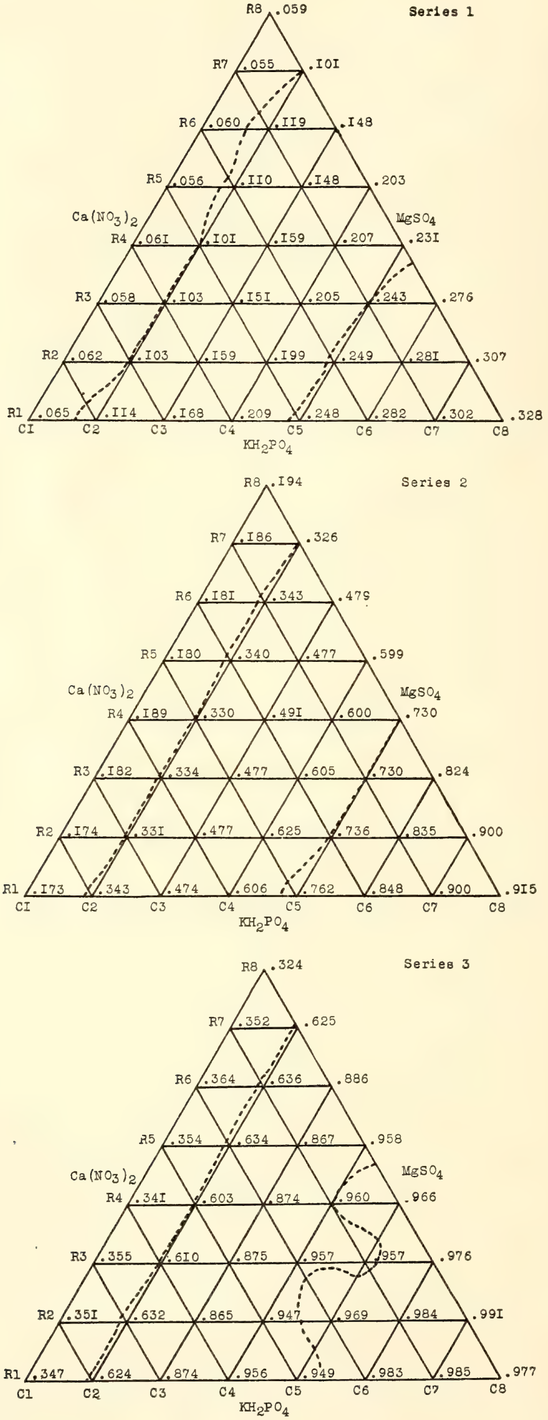


FIG. 3. Triangular diagrams showing relative yields from cultures of series 1 to 3 (group 1). Areas of low yields lie on the left margins of the diagrams, medium yields in the central regions between dotted lines, and high yields at the lower right of each diagram.

unbalanced condition of any of the solutions in any series which can be attributed directly to variations in the proportions of these two salts.

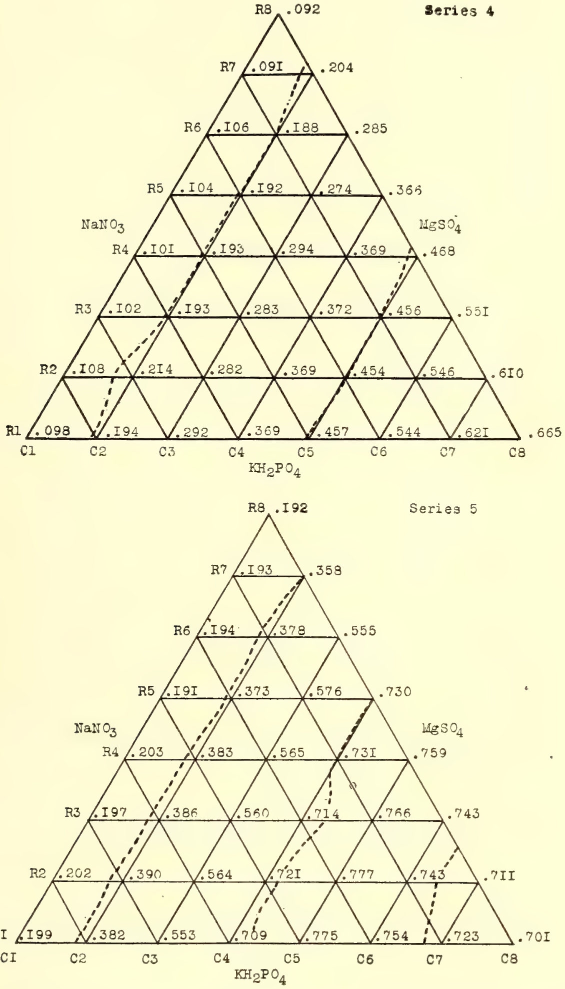


FIG. 4. Triangular diagrams showing relative yields from cultures of series 4 and 5 (group 2). Areas of low yields lie on the left margins of the diagrams, areas of medium yields in the central regions between dotted lines, and areas of high yields at the lower right of each diagram.

Steinberg (7) reported a very marked stimulative effect upon *Aspergillus niger* from high partial concentration of $MgSO_4$ and KH_2PO_4, but his results were obtained in a culture medium essentially free from iron. The addition of traces of iron gave similar stimulation, and he suggests that the increased yields in solutions with high partial concentrations of $MgSO_4$ might have been due to the heavy impurities in the $MgSO_4$ rather than to the salt itself. The stimulative effect of high partial concentrations of KH_2PO_4 he attributes to the increased acidity in these cultures. In media containing traces of iron and zinc an increase in acidity failed to give further stimulation. From these results of Steinberg it seems probable that the iron added to the media used in the experiments herein reported was sufficient to give "maximum stimulation," and that as a consequence the $MgSO_4$ and KH_2PO_4 salts could not produce the stimulative effect which they produced in Steinberg's iron-free medium.

In direct contrast to the indifferent effect of changes in the partial concentration of KH_2PO_4 and $MgSO_4$ is the effect of changes in the partial concentration of the nitrate salts. On the triangular diagrams of series 1 to 5 (figures 3 and 4) it will be observed that for each of the series there is a uniform increase in yield values in passing from the left to the right margin of the triangle representing the series; that is, as the proportion of the nitrate salt increases, there is a corresponding increase in the yield values. In series 1, for example, in those cultures in which $Ca(NO_3)_2$ furnished only 1/10 of the total salt concentration (C1 cultures) the yields are very low, varying between 0.055 and 0.065 gram. Where the $Ca(NO_3)_2$ is increased to 2/10 of the total concentration (C2 cultures) the yields are almost doubled, the values being between 0.101 and 0.119 gram for these cultures. Likewise, throughout the entire series an increase in $Ca(NO_3)_2$ is followed by a corresponding increase in yield. Thus, culture R1C8, which has the highest nitrate content of any culture in the series, has also the largest yield (0.328 gram). This same close correlation between the partial concentration of NO_3 and yield is shown for all the series, the only apparent exception being in the high-yielding cultures of series 3 and 5 in which the exhaustion of the sugar supply limited further growth.

The relation between the partial concentrations of NO_3 and yields is brought out very clearly in figure 5, which shows the dry weight yields plotted against NO_3 content of the cultures. In these graphs the abscissas represent grams of NO_3 per liter of culture medium, and the ordinates represent grams dry weight of fungus per culture. Each dry weight represents the average of all cultures of a single series containing equal quantities of NO_3.

It will be observed that the curves representing series 1, 2, and 3 (Ca group) are very nearly coincident, indicating that all cultures within this group which contain equal quantities of NO_3 have approximately the same yield values regardless of the total salt concentration or of the amounts of KH_2PO_4 and $MgSO_4$ present. This fact is brought out very clearly if a direct comparison is made between the yield values from cultures with the same NO_3 content but having different total concentrations. Thus, culture R1C8 of series 1, all the C2 cultures of series 2, and all the C1 cultures of series 3 contain approximately the same amount of NO_3 per liter, but differ widely in their total salt concentrations. Culture R1C8 of series 1 gave a yield of 0.328 gram. The average yield from the C2 cultures of series 2 is 0.333 gram, and from the C1 cultures of series 3 it is 0.348 gram. This agreement is extremely close when it is considered that the partial concentrations due to either KH_2PO_4 or $MgSO_4$ in these cultures vary from less than 0.1 atmosphere in series 1 to more than 3.0 atmospheres in series 3. A similar equality between dry-weight yields exists for all the cultures containing equal quantities of NO_3 in the group comprising series 1, 2, and 3. This same relation exists also in the group comprising series 4 and 5. The direction of the graph of each series with a given total salt concentration indicates an approximately linear relation between the dry-weight yields of the fungus and the proportions of NO_3 in the media.

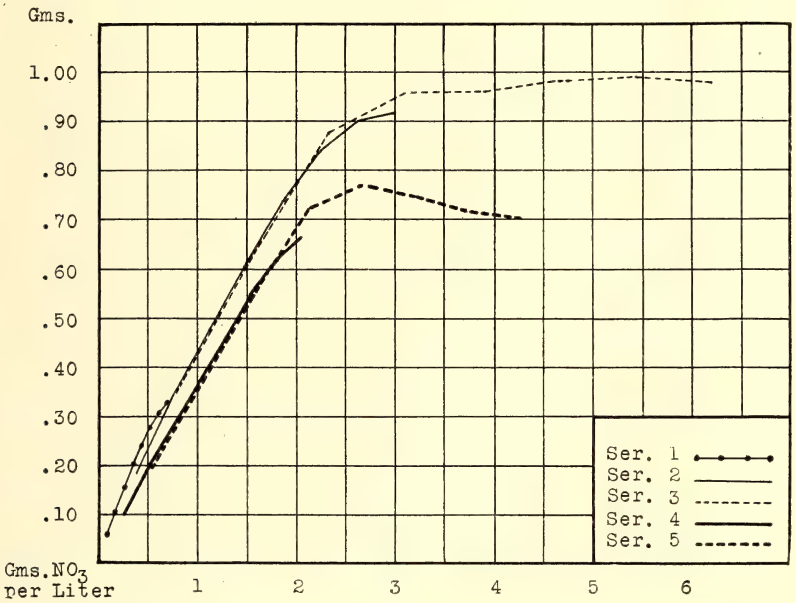


FIG. 5. Graph showing relation between dry-weight yields of *Aspergillus niger* and the NO_3 content of the nutrient solution.

It will be observed that the graphs of series 3 and 5 (fig. 5) break abruptly at the point where high yields are indicated. This sudden break itself, according to Blackman (1), indicates that a limiting factor for growth had entered at this point, and, as already pointed out, this is attributed to the exhaustion of the sugar content in these high-yielding cultures.

That growth in the two series in question was limited by the amount of sugar available in the cultures is clearly brought out in a series of eight duplicate cultures in which the sugar content was made to vary from a calculated osmotic concentration value of 1.0 atmosphere to one of 8.0 atmospheres, by increments of one atmosphere, the total salt concentration and the salt proportions remaining constant in all the cultures. The solution of culture R1C8 in series 3 was used in this test. This solution has a total salt-concentration value of 4.2 atmospheres, and the three salts KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$ are present in the proportions of 0.0710 m., 0.0063 m., and 0.0108 m., respectively.

The results of this test are shown in the graph of figure 6 in which the ordinates represent dry weights in grams and the abscissas represent concentrations of sugar in atmospheres. This graph brings out the fact that as the sugar concentration is increased (and therefore the amount of sugar per culture, since the amount of solution was the same in all the cultures, namely 50 cc.), the dry-weight yields were proportionately higher, thus showing a linear relation between the amounts of sugar employed and the yield values.

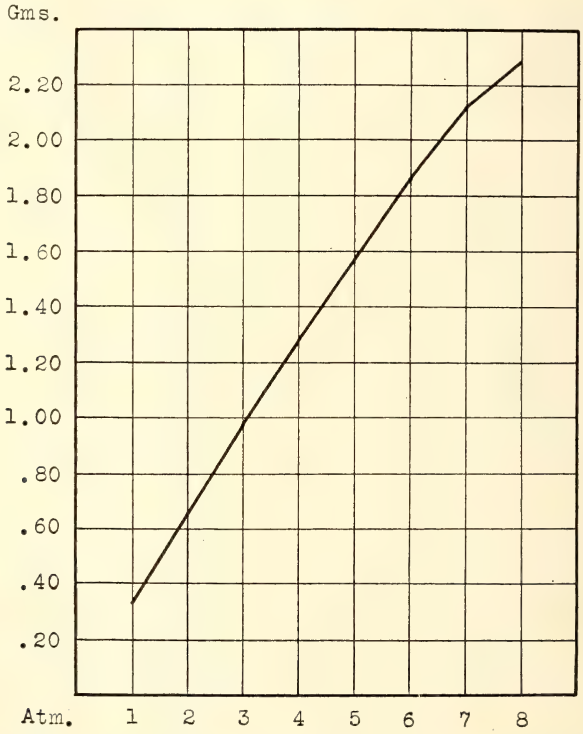


FIG. 6. Graph showing relation between dry-weight yields of *Aspergillus niger* and the sugar content of the nutrient solutions.

It is to be observed that the culture with a sugar content of 3 atmospheres gave an average yield of 0.974 gram, which is nearly the same as the yield from culture R1C8 of series 3 having the same composition, the yield obtained from this latter culture being 0.977 gram. However, the yields continued to increase proportionately with increase in sugar concentration up to a concentration of 8 atmospheres, at which concentration the dry weight yield was 2.283 grams. It is thus clear that with 50 cc. of solution having a sugar concentration of 3 atmospheres, the maximum yield which can be produced is between 0.9 gram and 1.0 gram, the point at which the curve of series 3 (fig. 5) breaks abruptly.

EFFECT UPON ASPERGILLUS NIGER OF SUBSTITUTING $NaNO_3$ FOR $Ca(NO_3)_2$ IN THE 3-SALT SOLUTIONS

A microscopic examination of the fungus from the cultures containing $Ca(NO_3)_2$ showed a slight precipitate of calcium oxalate crystals upon the hyphae. Not all of this deposit was removed by the washing process to which the fungus was subjected before drying. The amount of calcium oxalate crystals which remained after washing was not determined, so that it can not be stated definitely to what extent the dry weights were affected by it, but it is certain that increase in weight due to adhering crystals which remained after the washing process was small. However, since there is a possibility of the yield values of series 1, 2, and 3 being slightly higher than they should be, and since the formulae of media used for the growth of fungi do not ordinarily contain calcium nitrate, series 2 and 3 were repeated, substituting sodium nitrate in equivalent osmotic partial concentrations for the calcium nitrate in the various cultures of the corresponding series. These two series have already been referred to as series 4 and 5 of the $NaNO_3$ group, and the volume-molecular salt proportions of the solutions used are given in table 2. The yields from these two series are given in table 3 and plotted in the graphs of figure 5 in connection with those from the series of the $Ca(NO_3)_2$ group. The yields are also shown on the triangular diagrams of figure 4 which have already been considered.

A comparison of the graphs (fig. 2) shows at once that the yields from series 4 and 5 are uniformly much lower than are the corresponding yields from series 2 and 3 respectively. Thus, substituting in any culture $NaNO_3$ for $Ca(NO_3)_2$ in equivalent osmotic concentrations had the effect of reducing the yield very considerably. This great reduction in yield is largely accounted for by the fact that the nitrate content of the cultures in series 4 and 5 is only 68.3 percent of the NO_3 content in the corresponding cultures of series 2 and 3 respectively, this difference in the NO_3 content of the cultures being due, of course, to the difference in the composition and osmotic value of the nitrate salts employed. That this large difference in yields, however, is not entirely due to the difference in the NO_3 content of the cultures is clearly brought out by the graphs of figure 5. It will be observed that the graphs representing series 1, 2, and 3 lie throughout above the graphs of series 4 and 5, thus indicating that with equivalent amounts of NO_3 per culture (grams per liter of nutrient solution) the cultures containing $Ca(NO_3)_2$ gave uniformly higher yields than did the cultures containing $NaNO_3$.

The exact cause of these observed differences in yield is not clear. They are doubtless in part due to the deposit of calcium oxalate crystals on the hyphae in the $Ca(NO_3)_2$ series, but the differences are apparently too great to be entirely attributed to this cause. The reduced yield in the $NaNO_3$ group may in part be the result of a toxic influence of the Na, as seems to be indicated by the reduction in yield in the cultures containing the largest amounts of $NaNO_3$. This reduction in yield is indicated by the downward trend of the upper end of the curve representing series 5 (fig. 5). There is also a possibility that the absence of Ca has somewhat depressed the yields in the $NaNO_3$ group. Although it has been demonstrated that calcium is not essential to the growth of fungi, it may still be beneficial as an antagonizing influence in some such manner as has been suggested by Osterhout (5), who states that

> The classical researches of Pasteur and Raulin and the later work of other investigators have shown that calcium is not needed for the nutrition of fungi, and it is, therefore, omitted from culture solutions for these plants. This answers very well as long as the solutions are sufficiently dilute. I find, however, that when the concentration of the solution is increased it becomes toxic, and the addition of calcium then produces a remarkable improvement in growth. Calcium, therefore, has a protective value for fungi just as for other plants, though not needed for nutrition.

SUMMARY

Aspergillus niger was grown on three-salt solutions of total concentrations equivalent to 0.5, 2.1, and 4.2 atmospheres respectively. For each total concentration 36 solutions were made, representing all the possible combinations obtained by varying the partial concentrations of each of the salts by increments of 1/10 of the total concentration.

A number of solutions in which the salt proportions and total salt concentration remained the same, but with sugar concentrations varying from 1 to 8 atmospheres by increments of one atmosphere, were also tested.

1. In solutions all with the same salt proportions, an increase in total concentration gave a corresponding increase in yield.
2. The partial concentrations of KH_2PO_4 and $MgSO_4$ were varied within wide limits without in any way affecting the yields.
3. Yield in dry weight of fungus is approximately proportional to the amount of NO_3 present in the culture, whether this is produced by increasing total concentration and leaving salt proportions unchanged, or by changing salt proportions and leaving total concentration the same.
4. With a sugar solution having an osmotic concentration value of three atmospheres, the limit of growth in the $Ca(NO_3)_2$ cultures was between 0.9 gram and 1.0 gram, regardless of the amount of salts present.
5. In cultures with constant salt proportions and total salt concentrations but with varying sugar concentrations, the dry weights of fungus were very nearly proportional to the sugar concentrations of the cultures.

LABORATORY OF PLANT PHYSIOLOGY,
NEW JERSEY AGRICULTURAL EXPERIMENT STATION

LITERATURE CITED

1. **Blackman, F. F.** Optima and limiting factors. Annals of Bot. **19**: 281–295. 1905.
2. **Brenner, W.** Die Stickstoffnahrung der Schimmelpilze. Centralblatt Bakt. II, **40**: 555–647. 1914.
3. **Hibbard, R. P** Physiological balance in soil solution. Mich. Agr. Exp. Sta. Tech. Bull. **40**: 1–44. 1917.
4. **McCall, A. G.** Physiological balance of nutrient solutions for plants in sand cultures. Soil Sci. **2**: 205–253. 1916.
5. **Osterhout, W. J. V.** On nutrient and balanced solutions. Univ. Cal. Pub. Bot. **2**: 317–318. 1907.
6. **Shive, J. W.** A study of physiological balance in nutrient media. Physiol. Res. **1**: 327–397. 1915.
7. **Steinberg, R. A.** A study of some factors in the chemical stimulation of the growth of *Aspergillus niger*. Amer. Jour. Bot. **6**: 330–356; 357–372. 1919.
8. **Thom, C.** *Aspergillus niger* group. Jour. Agr. Res. **7**: 1–15. 1916.
9. **Tottingham, W. E.** A quantitative chemical and physiological study of nutrient solutions for plant cultures. Physiol. Res. **1**: 133–245. 1914.

SUGGESTIONS WITH RESPECT TO THE MEASUREMENT OF OSMOTIC PRESSURE

L. Knudson and S. Ginsburg

(Received for publication September 25, 1920)

The cryoscopic method for measuring osmotic pressure in plant tissues is at present the only convenient and probably, if the proper precautions are taken, the only reliable method for determining the osmotic pressure of plant tissues. Some of its advantages and limitations have been discussed by various men, among them Dixon and Atkins (2), and the technique of the operation has been considered by a number of men in this country, particularly by Gortner and Harris (4). While the methods of determining the freezing point of the expressed sap have been fairly well standardized and the necessary precautions have been generally followed by investigators, yet little attention has been given to the methods of freezing the tissue, or to the expression of the sap from tissues. It would be interesting also to have, if possible to obtain, data of osmotic pressures of tissues as determined by the freezing-point method and by the plasmolytic method. Dixon and Atkins have emphasized the necessity of freezing the tissue before expression of the sap, using liquid air to freeze the tissue. Gortner and Harris (4) considered the use of liquid air superfluous, but recently Harvey (5) has found that cabbage leaves, hardened to resist freezing, when frozen at −5° C. yielded a sap with a freezing point of −1.160° C.; when frozen with solid carbon dioxide, −1.630°, and when frozen with liquid air, −1.822° C. These differences are great enough to be significant. Harvey does not state specifically what pressures were used. He says that "pressures from 10–30 tons were used on a 2¼ inch ram."

In 1916, the authors began some work comparing the plasmolytic and the cryoscopic methods. Various circumstances have prevented thus far any continuation of the work, but the authors feel that the methods for expressing sap, and the relation of the pressure applied to the concentration of the expressed sap, make publication of the results desirable. This paper is concerned with the effect of the temperature at which the tissue is frozen and of the pressure applied on the freezing point of expressed sap. The paper includes also data on the osmotic pressures as determined by the plasmolytic and cryoscopic methods; a special apparatus is described for use in expressing sap, and suggestions are made for applying pressures of known values in expressing the sap.

METHODS

In the experiment here reported, two different species of plants were used: *Zebrina pendula* Schnizl, and *Iresine Herbstii* Hook. These plants were employed because a large supply was at hand and all were growing under the same environmental conditions and the plants of each species were of equal age. Furthermore, the presence of pigments in the cell sap makes easier plasmolytic determinations.

Plasmolytic determinations were made of the pigmented mesophyll cells of Iresine and of the pigmented cells of the lower epidermis of Zebrina. In making the determinations, free-hand cross sections of the leaves were used.

In the plasmolytic determinations, two solutions were used: calcium chloride and sucrose. Calcium chloride was used in preference to other salts for the reason that permeability, as shown by Osterhout (9, 10), True and Bartlett (12), and others, is generally decreased by calcium chloride. No correction was made for shrinkage. Shrinkage of cells would tend to make the osmotic pressure determination higher than the actual. Renner (11) believes that values obtained by the plasmolytic method would generally be lower than those obtained by the cryoscopic method, since in the latter, calculations are based on weight-normal solutions (gram molecules of solute per 1,000 grams of solvent), while in the plasmolytic method one generally uses volume-normal solutions (gram molecules of solute in 1,000 grams of solution). Throughout the plasmolytic experiments weight-normal solutions were used. Therefore, the values obtained by each method should be more comparable.

The osmotic-pressure values by the plasmolytic method were calculated, using 22.4 atmospheres as the osmotic pressure exerted by a weight-normal solution, and, for the cryoscopic method, osmotic pressure $= \Delta \times 22.4/1.86$. It might be more desirable to use the osmotic-pressure values obtained by Morse [see Findlay (3)] and his co-workers, but the values would be only slightly increased. In calculating osmotic pressures from the plasmolytic determinations made with calcium chloride, the formulas given by Livingston (8) were used. Dissociation was calculated from conductivity tables in Landolt-Börnstein (7).

All determinations were made of the leaves only. The plants were cut at 8 A.M. and placed with their cut ends in water for thirty minutes, so that the leaves would be in a turgid condition. In making the plasmolytic determinations, preliminary studies were previously made in order to obtain the approximate concentrations which would be isosmotic with the cell sap, and then, before each series of determinations, various dilutions of the two solutions were prepared, the concentrations of these ranging close to the anticipated threshold solution. In this way rapidity in determining the isosmotic concentration was obtained.

In expressing the sap for the cryoscopic method, the leaves were frozen either in salt-ice mixture or in liquid air. For the liquid-air treatment the leaves were strung on a thread and immersed in a Dewar flask containing the liquid air. The leaves were removed when the liquid air stopped boiling, indicating that the leaves had assumed the temperature of liquid air.

The leaves when removed were very brittle, and they were immediately placed in a wide-mouthed glass-stoppered bottle in order to prevent condensation of water on their surfaces. In freezing the leaves with ice-salt mixture, the leaves were first placed in a glass-stoppered wide-mouthed bottle and then immersed in the freezing mixture until the leaves were frozen solid. The time of exposure was one hour. The bottle was then removed and carefully rinsed to free the outside surfaces of any adhering salt, and then wiped dry. The amount of leaves used in every case was 50 grams of fresh leaves, which for Zebrina is approximately 125 leaves and for Iresine 250 leaves.

For expressing the sap a special apparatus was constructed (fig. 1). This consists of a steel cylinder, internal diameter four inches, and a closely fitting solid steel piston. At the base of the interior of the cylinder, around the circumference, is a small groove 1/16 inch deep and 1/16 inch wide. There is an opening from this groove leading to the exterior, which is fitted with a steel tube of a diameter of 1/8 inch. The purpose of the groove is to prevent the juice from being forced upwards between the cylinder and the piston and also to prevent the tissue from being forced outwards through

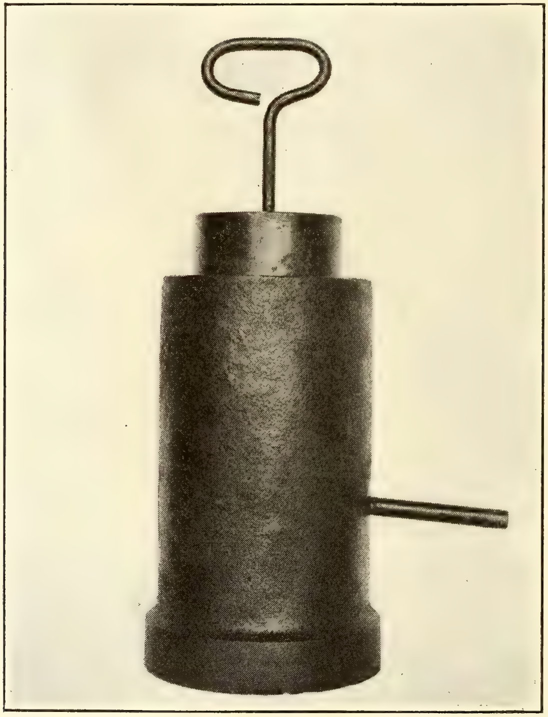


FIG. 1. Apparatus for expression of sap. The handle is unscrewed before the apparatus is placed in the press.

the tube. The leaves were wrapped in several layers of washed muslin before being placed in the cylinder. The cylinder and piston apparatus may, of course, be constructed of any size which would be convenient.

For applying pressure to the piston, use was made of materials-testing machinery of the College of Engineering. The one used gave a total pressure up to 50,000 pounds, while with the others available, pressures up to 400,000 pounds could be obtained. The advantage of these machines is that absolutely known pressures can be obtained, and they are available in all engineering laboratories.

The freezing points were determined by the Beckman apparatus. The sap was allowed to undercool to −1.0° C., when it was inoculated by means of a platinum needle with crystals of hoar frost. Undercooling, therefore, was the same in all cases and practically negligible as to its influence.

EXPERIMENTS

In this experiment, the influence of the method of freezing and of the amount of pressure applied to the tissue is noted. Leaves of Iresine were used. Total pressures of 50,000 and 10,000 pounds were used, and the tissue was frozen either by liquid air or by the salt-ice mixture. The data given in table 1 record the osmotic pressures as calculated from the depression of the freezing point.

TABLE 1

Method of Freezing	Pressure Applied to Leaves	Osmotic Pressure in Atmospheres
Ice and salt	50,000 lbs.	5.674
Liquid air	50,000 lbs.	5.714
Ice and salt	10,000 lbs.	4.571
Liquid air	10,000 lbs.	4.932

It is apparent from the data that the amount of pressure applied is an important factor. The difference in the osmotic pressure determined from the sap expressed at 10,000 pounds and that expressed at 50,000 is with either method of freezing close to one atmosphere. On the other hand, the method of freezing the tissue previous to extraction of the sap shows but little difference in the osmotic-pressure values obtained. The difference is greater when a pressure of 10,000 pounds is used than when a pressure of 50,000 pounds is employed.

Similar data were obtained with the Iresine in other experiments and also with *Zebrina pendula.*

Comparison of the plasmolytic and cryoscopic methods in determining osmotic pressure. Tables 2 and 3 give the osmotic pressures as determined by the plasmolytic and cryoscopic methods. Table 2 gives the data for *Zebrina pendula*, and table 3 for Iresine. The four series of determinations for each species were made on different days. For the cryoscopic determination, the leaves were frozen by immersion in liquid air and extraction of the sap was made under a pressure of 50,000 pounds; conditions which would yield the highest values.

TABLE 2. *Zebrina pendula*

No. of Exp.	Osm. Pr. in Atm. with Sucrose	Osm. Pr. in Atm. with $CaCl_2$	Average	Cryoscopic
No. 1.....	4.233	4.480	4.356	5.173
No. 2.....	4.121	4.650	4.385	6.425
No. 3.....	4.188	4.704	4.446	5.714
No. 4.....	4.211	4.569	4.390	5.293

TABLE 3. *Iresine*

No. of Exp.	Osm. Pr. in Atm. with Sucrose	Osm. Pr. in Atm. with $CaCl_2$	Average Atm. Plasmolytic	Atm. Cryoscopic
No. 1.....	6.720	7.078	6.899	8.300
No. 2.....	6.496	7.101	6.798	9.443
No. 3.....	6.944	6.809	6.876	7.981
No. 4.....	6.329	6.918	6.623	8.016

A comparison of the various data indicates that the cells must permit of the entrance of $CaCl_2$ more readily than the sucrose, since the osmotic-pressure values as determined are higher when the plasmolyzing solution is $CaCl_2$ than when the solution is sucrose. The values as determined by the cryoscopic method are in all cases higher than the osmotic-pressure values as obtained from the plasmolytic method. In the case of *Zebrina pendula*, hundreds of determinations made during the past ten years of the osmotic pressures of epidermal cells by the plasmolytic method, using various salts and sugars, give values which range between 4.0 and 4.5 atmospheres. It would appear, therefore, that in the case of *Zebrina pendula* the values as obtained by the plasmolytic method are not far from being the true values for the cells observed. It should be pointed out, however, that the plasmolytic values were obtained entirely from pigmented cells of the lower epidermis, and it is not improbable that the osmotic pressure of the chlorophyllous cells is greater than that of the pigmented cells. Furthermore, the guard cells of the stomates, as pointed out by Iljin (6), may show a much higher osmotic pressure than the adjacent cells, the differences observed being as high as 80 atmospheres. In our laboratory, R. A. Wiggans has found by the plasmolytic method, differences not to exceed 7 atmospheres for Iresine and 6 atmospheres for *Zebrina pendula*.

Another possible explanation to account for the great difference is that by the cryoscopic method the sap is expressed from the conducting tissue as well, and it is not improbable that the concentration of solutes in the conducting system may be in excess of that of the mesophyll cells. This idea is supported by the evidence of Davis, Daish, and Sawyer (1), who found that the ratio of hexoses to sucrose is higher in the midrib and stalks than in the remainder of the leaf, and also that total sugars are actually higher.

REMARKS

As pointed out previously, Harvey (5) noted that the method of freezing the tissue affected the concentration of the sap. Assuming that the same pressure was used by him in expressing the sap from leaves frozen by the different methods, it follows that the investigator must exercise some discretion in the method employed for freezing the tissue.

It seems to the writers, however, that the amount of pressure applied is more important than the method of freezing. What amount of pressure should be used would depend upon the character of the plant tissue under investigation. It would seem desirable to use that pressure which would yield a sap of the greatest concentration, and the investigator should state specifically the pressure employed. Furthermore, if tissues of unlike character are under investigation, as, for example, parenchymatous and woody tissues, it would seem that a greater pressure would be needed for the woody tissue than for the parenchymatous tissue in order to give comparable results. In other words, with a parenchymatous tissue a pressure of 10,000 pounds might yield a sap containing 95 percent of the solutes of the original sap, while for a woody tissue the same percentage of solutes might be obtained only with a pressure of 50,000 pounds. It seems to the writers, therefore, that in any investigation involving the determination of osmotic pressures of plant tissues, preliminary experiments are essential in order to determine the most desirable methods of freezing and the amount of pressure to be applied.

SUMMARY

1. A piston-cylinder apparatus is described for use in the expression of cell sap.

2. Recommendation is made with respect to the use of standard materials-testing machinery where definite pressures are available.

3. Experiments show that a pressure of 50,000 pounds yields a more concentrated sap than a pressure of 10,000 pounds.

4. No great differences were found in the concentration of the sap expressed from leaves frozen with liquid air or with an ice-salt mixture.

5. Considerable differences were observed between the osmotic pressure as determined by the plasmolytic and by the cryoscopic methods.

LABORATORY OF PLANT PHYSIOLOGY,
CORNELL UNIVERSITY, ITHACA, NEW YORK

BIBLIOGRAPHY

1. **Davis, W. A., Daish A. J., and Sawyer, G. C.** Studies of the formation and translocation of carbohydrates in plants. Jour. Agr. Sci. 7: 255–314. 1916.
2. **Dixon, H. H.** Transpiration and the ascent of sap in plants. 1914.
3. **Findlay, A.** Osmotic pressure. 1919.
4. **Gortner, R. A., and Harris, J. A.** Notes on technique of determinations of freezing points of vegetable saps. Plant World 17: 49–53. 1914.

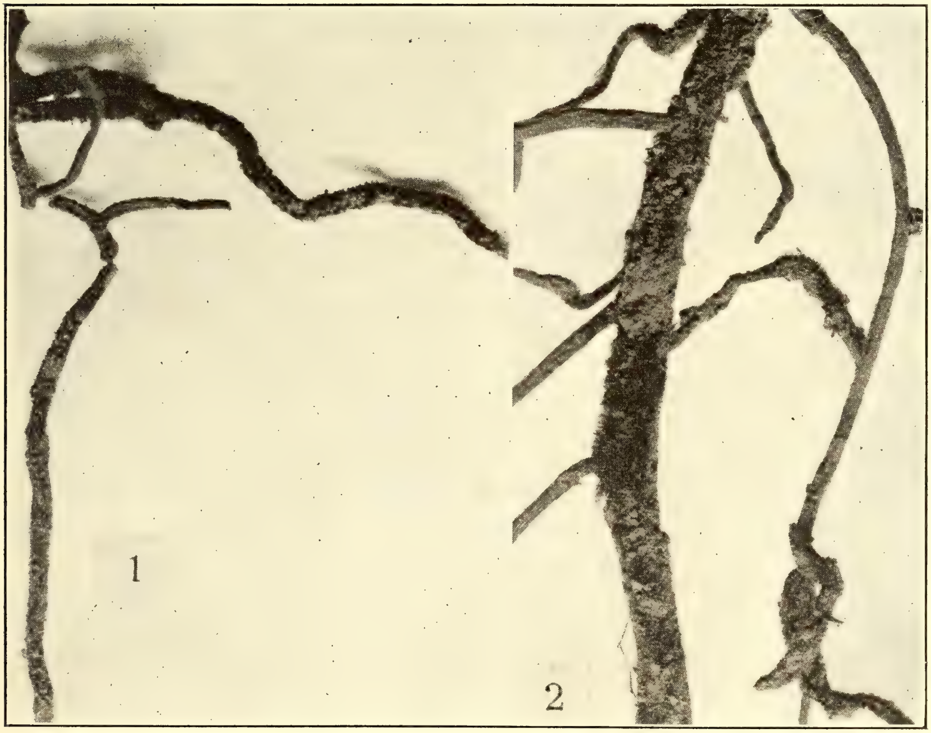
5. **Harvey, R. B.** Hardening process in plants and developments from frost injury. Jour. Agr. Res. **15**: 83–111. 1918.
6. **Iljin, W. S.** Die Regulierung der Spaltöffnungen mit der Veränderung des osmotichen Druckes. Beih. Bot. Centralbl. **32**: 15–36. 1914.
7. **Landolt, H., Börnstein, R., and Roth, W.** Physikalisch-chemische Tabellen. 1912.
8. **Livingston, B. E.** Rôle of diffusion and osmotic pressure in plants. 1903.
9. **Osterhout, W. J. V.** The permeability of living cells to salts in pure and balanced solutions. Science n. ser. **34**: 187–189. 1911.
10. **Osterhout, W. J. V.** A comparative study of permeability in plants. Jour. Gen. Physiol. **1**: 299–304. 1919.
11. **Renner, O.** Ueber die Berechnung des osmotischen Druckes. Biol. Centralbl. **32**: 486–504. 1912.
12. **True, R. H., and Bartlett, H. H.** The exchange of ions between the roots of *Lupinus albus* and culture solutions containing one nutrient salt. Amer. Jour. Bot. **2**: 256–278. 1915.

THICK-WALLED ROOT HAIRS OF GLEDITSIA AND RELATED GENERA

W. B. McDougall

(Received for publication September 30, 1920)

A few years ago my attention was called by the late Professor T. J. Burrill to the peculiar appearance of the surface roots of *Gleditsia triacanthos*, the honey locust, growing in the "Forestry" at the University of Illinois. These roots, which were abundant and easily obtained, when examined with the naked eye looked somewhat like ectotrophic mycorrhizas. It was noticed at once, however, that instead of the coral-like clusters of short, stubby roots characteristic of ectotrophic mycorrhizas the branching was normal, and the peculiar-appearing portion extended several inches back from the ends of the roots and in fact included practically all of the smaller parts of the root system. The color was rather dark brown. Under a lens



Figs. 1, 2. Roots of *Gleditsia triacanthos* covered with thick-walled root hairs.

these roots presented the appearance that is seen in figures 1 and 2: bristling with projections which I still thought to be due to a fungus. A cross section examined under the microscope, however, showed at once that these projections are thick-walled root hairs; figure 3 being a camera lucida drawing of one of these hairs. The actual thickness of the walls of these hairs, as measured with a Spencer Lens Co. screw micrometer, averages approximately 2 μ. This is about four times as thick as the walls of the root hairs of barley seedlings grown in petri dishes on filter paper. It is also about four times as thick as the walls of the newly formed hairs on the roots of honey locust seedlings grown in soil, as will be brought out later.

The hairs do not average very long, only about four tenths of a millimeter, but they are stiff and rigid and do not shrivel when exposed to the air. The photographs shown in figures 1 and 2 were taken from specimens that had lain unprotected on my desk for more than a month, during which time they had not shriveled in the least.

The chemical composition of the walls has not been determined. It is certain that chemical changes take place during the thickening of the walls, but repeated tests with phloroglucin and hydrochloric acid failed to detect any lignification while a cellulose reaction was finally obtained by allowing sections to remain in chloriodide of zinc solution for twenty-four hours. It has also been impossible to determine whether these hairs contain living protoplasm, for the walls are so thick and so dark-colored that it is not possible to see through them.

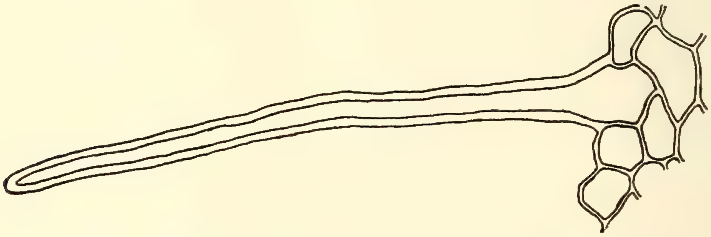


FIG. 3. A thick-walled root hair of *Gleditsia triacanthos*.

During the summer of 1919 I examined the roots of a large number of *Gleditsia triacanthos* trees in various localities and in very diverse habitats, the habitats ranging from the climax upland forest of Champaign county, Illinois, to the typical bottomland forests along the Vermillion River in Vermillion County, Illinois. To my surprise I found that the presence of thick-walled root hairs is a constant character of this species. I have not been able to find a honey locust tree on whose roots the hairs were not present in abundance. In order to determine whether the hairs are present on more than the superficial roots, a hole was dug near a honey locust tree in the "University Woods" (a sixty-acre tract of climax forest belonging to the University of Illinois and situated four miles northeast of Urbana, Illinois), and the roots were examined to a depth of four feet. Thick-walled root hairs were found as abundantly at that depth as near the surface. In some cases the smaller parts of the root system are completely covered with root hairs while in other cases there are patches which are entirely free from hairs. These latter are probably due to the fact that the movements of the roots in the soil due to growth often break off the hairs.

Early in February, 1920, some seeds of *Gleditsia triacanthos* were obtained and planted in the greenhouse. After seedlings had appeared, the root systems of some of them were examined at the end of one week, three weeks, four weeks, eight weeks, fourteen weeks, and twenty-one weeks. The following excerpts from my notes will serve as a report on this part of the work:

Feb. 17. Seedlings one week old. The root (tap root) is 7 cm. long and unbranched. It is abundantly covered with root hairs throughout its entire length. Near the tip these hairs are whitish or colorless and have very thin walls. They contain protoplasm and look very much like the root hairs of other plants. Toward the base of the root the hairs are becoming brown and the walls are somewhat thickened.

March 2. Seedlings three weeks old. The total height of the plant above ground is about 14 cm. The tap root is from 10 to 12 cm. long and has 10 to 15 branches varying from 1 mm. to 2 cm. in length. The entire root system is clothed with root hairs. The thickness of the walls of the oldest and brownest hairs is now about 0.95 μ, while the walls of the young hairs near the tips of the roots are only about 0.5 μ thick.

March 9. Seedlings four weeks old. The longest secondary root found at the age of three weeks was 2 cm. The longest at four weeks is 5 cm. These secondary roots, therefore, may grow as much as 3 cm. in a week, or more than 4 mm. per day. The youngest hairs are usually found about 4 to 5 mm. from the tip, while at 12 mm. from the tip the hairs are beginning to turn brown. Therefore, they probably remain colorless only about two days.

April 6. Seedlings eight weeks old. The thickness of the walls in the oldest hairs is now about 1.5 μ.

May 18. Seedlings fourteen weeks old. The walls of the oldest hairs have now reached the full thickness of approximately 2.0 μ. The epidermis has ruptured in several places on the older parts of the root system, and the root hairs have disappeared from parts.

July 6. Seedlings twenty-one weeks old. The entire length of the root system is now about 27 cm., and branches are numerous. Root hairs are present on most of the root system. They are very abundant as much as 15 cm. from the tips of some of the roots. On the first 7 or 8 cm. below the surface of the soil the epidermis has ruptured and many of the hairs have disappeared. Many still remain, however, even in this region, though they are often broken. There are other places on the root system where root hairs are entirely lacking, probably because of movements of the roots within the soil which break them off. It is apparent that in general the hairs remain for as long a time as the epidermis.

During the spring and summer of 1920 the roots of a number of trees of *Gymnocladus dioica* and *Cercis canadensis*, both of which are closely related to Gleditsia, were examined. It was found that both of these trees produce thick-walled root hairs similar to those of *Gleditsia triacanthos* but by no means so abundantly as in the latter species. In Cercis they are even less frequent and less evident than in Gymnocladus.

Discussion

Structures such as the thick-walled root hairs under discussion are commonly associated with xerophytic conditions, and at first it was thought that perhaps desiccation would explain their presence. When they were found in various types of habitats including bottomland forests, however, and when they were found deep in the soil as well as in the superficial layers, the idea that their presence could be explained as due directly to desiccation was quickly dispelled. However, *Gleditsia triacanthos* has a rather wide specific range of tolerance with respect to habitat. In Illinois it occurs most commonly in bottomland forests along streams, where it is a characteristic tree. But it is also a characteristic tree of the dry barrens of Kentucky and of dry hills elsewhere. For this reason, it is the opinion of the writer that these thick-walled hairs, together with the thorns which are also so characteristic of this species, are relics of a time when the tree grew only under more arid conditions. (It should be added here that *Gleditsia triacanthos inermis*, which lacks the thorns, produces thick-walled root hairs as abundantly as the species). In the case of Gymnocladus and Cercis, both of which have a lesser specific range of tolerance with respect to habitat than has Gleditsia, there have probably been greater constitutional changes during the course of evolution, and they have retained the characteristics due to an arid climate to a much lesser extent.

As previously stated, my observations indicate that in general, except when broken off by growth movements of the roots in the soil, the root hairs remain for as long a time as the rest of the epidermis. In some cases this may be only a few weeks while in other cases it may be several months. McDougall[1] has shown that the roots of trees grow at any time during the year when they can absorb a sufficient amount of water. Some growth would take place, therefore, and some root hairs would be formed late in the growing season. These would undoubtedly persist through the winter and into the next year until such time as there was sufficient growth to rupture the epidermis and finally cause it to be cast off. Indeed, it is not improbable that in some cases these hairs could rightly be spoken of as perennial.

Harrison and Barlow[2] state that *Gleditsia triacanthos*, *Gymnocladus dioica*, and *Cercis canadensis* never have root nodules (bacterial) but that mycorrhizas are always present on these species. I have never been able to find mycorrhizas on any of the leguminous trees, and it is my opinion that the roots bristling with thick-walled root hairs were mistaken by Harrison and Barlow for mycorrhizas. The absence of both root nodules and mycorrhizas from the roots of these trees is probably to be explained by the fact that the walls of the root hairs become thick and hard so quickly that the parasitic organisms are unable to gain an entrance. It will be recalled that the three trees under consideration all belong to the subfamily Caesalpinioideae. The native species of Cassia, the only other genus of this subfamily represented in our flora, are all herbaceous. They produce root nodules, and so far as I have examined them they do not produce thick-walled root hairs. *Robinia Pseudo-Acacia*, the common locust, belongs to the subfamily Papilionoideae. It also produces nodules and does not have thick-walled root hairs.

---

[1] McDougall, W. B. The growth of forest tree roots. Amer. Jour. Bot. **3**: 384–392. 1916.

[2] Harrison, F. C., and Barlow, B. The nodule organism of the Leguminosae—its isolation, cultivation, identification and commercial application. Centralbl. Bact. **19**: 264–272; 426–441. 1907.

Summary

1. The root hairs of *Gleditsia triacanthos* become thick-walled and brown in color within a few days after they are produced. This takes place regularly in all sorts of habitats.

2. These thick-walled root hairs persist as long as the root epidermis, whether this be for a few weeks or for several months, unless they are accidentally broken off by root movements in the soil.

3. The root hairs of *Gymnocladus dioica* and *Cercis canadensis* sometimes become thick-walled and brown, but this phenomenon is not nearly so characteristic of these two species as it is of *Gleditsia triacanthos*.

4. The thick-walled root hairs are considered xerophytic structures and are believed to be relics of a time when the species which possess them grew only under xerophytic conditions.

5. The trees which have thick-walled root hairs produce neither bacterial nodules nor mycorrhizas. This is probably due to the inability of the parasitic organisms to enter the roots through root hairs with thickened walls.

University of Illinois,
Urbana, Illinois

A SIMPLE METHOD FOR GROWING PLANTS

J. M. Brannon

(Received for publication December 6, 1920)

In growing plants under sterile conditions, investigators have employed either agar cultures or some other substratum of solid or semi-solid character placed in culture tubes, or else they have used water or soil cultures. In the water or soil cultures the roots only are maintained under sterile conditions; the leaves and stems being exposed to an unconfined atmosphere. In the course of certain experiments which are to be reported at a future date, it was found that neither the agar-culture method nor the water-culture method was satisfactory for growing green plants in the dark.

In the course of investigations on the organic nutrition of plants, it was noted at various times that seeds would germinate and seedlings would grow even when entirely immersed in a liquid medium. As a result of these incidental observations, it was decided to test the possibility of using such liquid cultures for the investigations. Striking successes were obtained, and the superiority of this method for growing plants in the dark over the agar method or the water culture methods hitherto used was at once apparent.

In a flask or culture tube, the size depending upon the plants to be grown and upon the duration of the experiment, is placed the culture solution. The depth of the solution should not exceed six centimeters. The vessels are plugged with cotton and then autoclaved. The seeds to be sown are then sterilized and the desired number sown in the culture solution. In the work here reported, the seeds were sterilized by the calcium hypochlorite method of Wilson.[1] This method of growing plants has been used with flax, alfalfa, corn, pea, and timothy. These were all grown in the dark. The pea and alfalfa have been grown for nine months in the dark when supplied with sugar. The method may also be used for growing plants in the light.

Table 1 gives data from an experiment with timothy grown in the dark,

Table 1

Solution Used	Weight of Individual Plant Exposed in Gms.	Average Length of Plants in Cm.	No. of Leaves
Pfeffer's + sugar	0.0180	13.5	4
Pfeffer's + sugar	0.0172	15.	
Pfeffer's + sugar	0.0184	16.	2
Pfeffer's alone	0.0071	6.5	

---

[1] Wilson, J. K. Calcium hypochlorite as a seed sterilizer. Amer. Jour. Bot. **2**: 420–427. 1915.

and in figure 1 is shown one of the cultures. The nutrient solution contained 2 percent sucrose.

The special advantage of this method is in the fact that the plants used will live and grow for a much longer period of time than by the other methods.

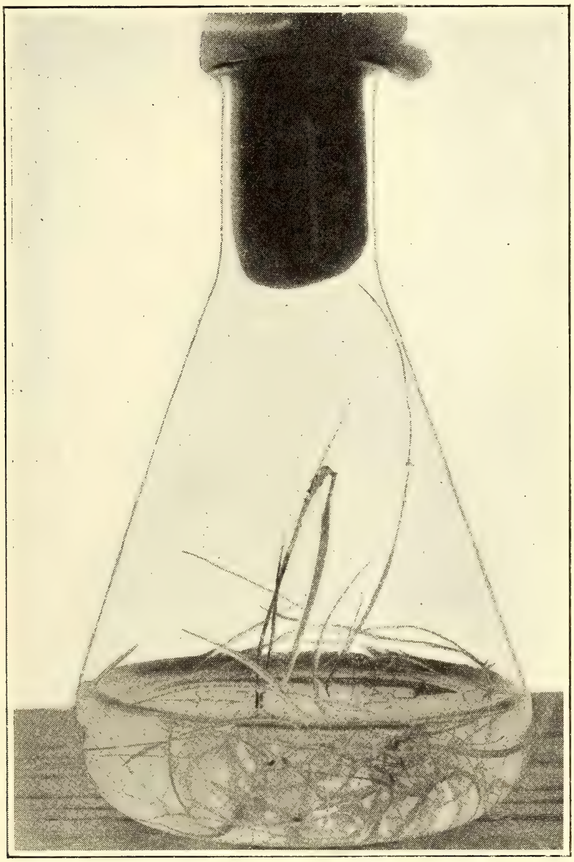


FIG. 1. Timothy grown for three weeks in the dark on Pfeffer's nutrient solution plus 2 percent sucrose.

It would seem, in the case of plants grown in the dark, that the sugars are either too slowly absorbed by the roots or that conduction of the sugars is too slow to satisfy the needs of the plant for organic matter. This idea has been suggested by Knudson and Lindstrom[2] in their work on albino corn. When a portion of the stem of the plant is also immersed, the stem probably absorbs sugars and so the needs of the plant are more nearly met.

Another advantage over the agar method is the greater ease of analyzing the solution. In the agar method the agar must first be removed before the sugar determination can be made. Adsorption phenomena inadvertently play a part in the precipitation of agar, and thus another source of error is introduced.

---

[2] Knudson, L., and Lindstrom, E. W. Influence of sugars on the growth of albino plants. Amer. Jour. Bot. **6**: 401–405. 1919.

Laboratory of Plant Physiology,
Cornell University

BOTANICAL SOCIETY OF AMERICA

Reorganized 1906, uniting the Botanical Society of America (1893), the Society for Plant Morphology and Physiology (1896), and the American Mycological Society (1903).

OFFICERS FOR 1921

President: C. E. Allen
University of Wisconsin

Vice-President: H. C. Cowles
University of Chicago

Secretary: J. R. Schramm
Cornell University, Ithaca, N. Y.

Treasurer: Irving W. Bailey
Bussey Institution, Harvard University

Councilors:

N. L. Britton
New York Botanical Garden

William Trelease
University of Illinois

J. C. Arthur
Purdue University

Any one actively interested in botanical work is eligible for membership. Candidates for membership are recommended by three members, not members of the Council, on blanks to be obtained from the Secretary.

The annual dues are $5.00, including subscription to the American Journal of Botany. Candidates proposed for membership, and approved by the Council, may receive one copy of the Journal at $4.00 per year pending their election at the winter meeting of the Society.

Do Our Greenhouses Really Differ So Much From Others

Outwardly most folks are mostly alike.

So are most greenhouses.

But inwardly—that's quite a different matter.

Just as the way a man gives attention to the little things—tells you the kind of a man he is; just so the care and particularity given to the little details in a greenhouse, tells you the kind of a house it is.

Some owners have accused us of being rather fussy about our work, intimating that we could cut corners and get the work done quicker.

And they are right.

But you'll agree that we are also right in refusing to do it.

That then might be one of the differences in which our greenhouses differ.

Hitchings and Company

Home Office and Factory: ELIZABETH, N. J.

NEW YORK
101 Park Ave.

BOSTON-9
294 Washington St.

Vol. VIII. APRIL, 1921 No. 4

AMERICAN JOURNAL OF BOTANY

OFFICIAL PUBLICATION OF THE
BOTANICAL SOCIETY OF AMERICA

Smithsonian Institution
MAY - 2 1921
National Museum

CONTENTS

The morphology and anatomy of Rhus diversiloba . . . . James B. McNair 179
Distribution of the Malvaceae in southern and western Texas
Herbert C. Hanson 192
Note on the histology of grain roots . . . . Grace A. Dunn 207
North American pipers of the section Ottonia . . . . . . William Trelease 212
Monocarpy and pseudomonocarpy in the Cycadeoids . . . . G. R. Wieland 218

PUBLISHED
IN COOPERATION WITH THE BOTANICAL SOCIETY OF AMERICA
BY THE
BROOKLYN BOTANIC GARDEN
At 41 North Queen Street, Lancaster, Pa.

Entered as second-class matter February 21, 1914, at the post office at Lancaster, Pennsylvania,
under the act of March 3, 1879.

AMERICAN JOURNAL OF BOTANY

DEVOTED TO ALL BRANCHES OF BOTANICAL SCIENCE

ESTABLISHED 1914

EDITED BY A COMMITTEE OF THE
BOTANICAL SOCIETY OF AMERICA

EDITORIAL COMMITTEE

C. E. ALLEN, Editor-in-Chief,
University of Wisconsin

WILLIAM CROKER,
University of Chicago

C. STUART GAGER, Business Manager
Brooklyn Botanic Garden

R. A. HARPER,
Columbia University

L. R. JONES,
University of Wisconsin

ORLAND E. WHITE,
Brooklyn Botanic Garden

JACOB R. SCHRAMM,
Cornell University

C. L. SHEAR, *Bureau of Plant Industry*
(Representing American Phytopathological Society)

The Journal is published monthly, except during August and September. Subscription price, $6.00 a year. Single copies 75 cents. Back numbers, 75 cents each; $6.00 a volume, postage extra. Postage will be charged to all foreign countries, except Mexico, Cuba, Porto Rico, Panama Canal Zone, Republic of Panama, Hawaii, Philippine Islands, Guam, Samoan Islands, and Shanghai. Postage to Canada, 20 cents a volume on annual subscriptions; to all other countries in the Postal Union, 40 cents a volume on annual subscriptions.

The pages of the Journal are open to members of the Botanical Society of America, or to candidates approved for membership.

Manuscript offered for publication should be typewritten, and should in all cases be submitted to the Editor-in-Chief.

Papers are limited to 20 pages in length, exclusive of plates, except that additional pages may be arranged for at cost rate to authors (approximately $3.00 a page).

Proofs should be corrected immediately on receipt, and returned to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y.

Illustrations, other than zinc etchings, and tabular matter should, in general, be restricted to approximately ten percent of the number of pages of text. Authors may be charged at cost rates for illustrations and tabular matter in excess of that amount (approximately $6.40 a page for tabular matter; 15 cents per square inch for half-tones).

Separates should be ordered when proof is returned. Ffty copies without cover will be supplied free; cover and additional copies at cost.

Remittances should be made payable to American Journal of Botany. Ten cents must be added to all checks not drawn on New York City banks.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

Correspondence concerning editorial matters should be addressed to Prof. C. E. Allen, University of Wisconsin, Madison, Wisconsin.

Business correspondence, including notice of change of address, and directions concerning reprints, should be addressed to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y., or 41 North Queen Street, Lancaster, Pa.

AMERICAN
JOURNAL OF BOTANY

VOL. VIII APRIL, 1921 No. 4

THE MORPHOLOGY AND ANATOMY OF RHUS DIVERSILOBA

JAMES B. McNAIR

(Received for publication November 2, 1920)

Rhus diversiloba T. & G. may be either a low deciduous shrub or a high climbing vine as it readily adapts itself to local conditions. In sunny exposures it most frequently occurs as a low shrub 3 to 4 feet high. One instance is known, however, of a tree-like plant, 14 feet high with a diameter at its base of 6 inches. In shady locations it more frequently assumes a vine-like form and by means of its aerial rootlets ascends the trunks of trees to a height of 15 to 20 feet.

The new wood has pale grayish bark, is light in weight, brittle, and contains much pith. The ends of the young shoots and the petioles are usually red-brown in color. In the early spring groups of plants may be distinguished at a distance before fully leafing out by this red-brown of the young stems and leaves. The branches gradually become woody with the accumulation of successive annual rings, the bark thickens, roughens, and becomes the abode of many lichens and mosses. The bark on the old wood is brown-gray, furrowed lengthwise, has horizontal rows of wart-like lenticels, and on dead limbs peels off laterally, not spirally. The large branches have but few small lateral branches. In its shrubby form no difference can be observed between the height of the male and that of the female plants, nor can any noticeable difference be determined in the angles which the branches subtend to the trunk. The leaflets, too, generally have similar shapes on both plants.

Flower panicles do not develop on the ends of young shoots, but form on the sides. The apical bud makes a growth of 4 to 5 centimeters per year. It requires about four weeks for the full development of a leaf. The flower and leaf buds begin to expand simultaneously, but the leaves soon expand more rapidly and consequently some of the leaves reach maturity before the flowers open. The expanding leaves are very tender and turgid, and sap flows quickly out of injured stem or leaf areas. These leaves are arranged alternately on the stem and have a phyllotaxy of 8/21.

The leaf scars are triangular in outline. The swelling where the leaf is attached may have the function, by its growth, of turning the leaf to a better exposure.

[The **Journal** for March (8: 127–178) was issued April 3, 1921.]

The Leaf

The glossy, dark green leaflets are deepest in color when in the sun, pale underneath, generally 3 in number, although sometimes 5, orbicular to ovate or oblong-ovate, undulate or plane, entire or variously lobed, segmented or toothed, 1 to 4 inches long. The 5-leaflet variety, according to Brandegee (3) is quite common on the Santa Barbara Islands. Leaves having 5 leaflets are also found on plants which have a majority of the 3-leaflet kind. Leaflets are singularly variable in size, outline, and segmentation, even on the same plant. This fact constitutes one of the most remarkable features of the plant and is the principal basis for its differentiation from *Rhus Toxicodendron* L. Leaf tracings (21) made from mature leaves collected by the writer at Berkeley, California, on September 27, 1916, were taken from plants within a radius of 100 feet, all of which were enjoying the same soil and exposure and had no apparent cause for such marked differences in leaf shape.

Leaves in the sun differ from those in the shade, not only as regards color but also in several structural details. The young leaves are covered with hairs, which dry out and fall off as the leaves become fully matured. These hairs are apparently more frequent on leaves exposed to the sun than on those in the shade. Other differences will be described later.

In autumn, as in spring and summer, the plant is singularly attractive, its leaves turning many shades of red, yellow, and brown. This color change may be induced in mature leaves in midsummer by certain insect injuries, by attacks of fungi, or by an interference with the flow of sap caused by twisting the stem. There is no apparent difference between the leaves of male and female plants in this respect. Some plants, however, particularly those in the shade, may have all their leaves yellow. Conversely, red leaves seem to be peculiar to plants of sunny exposure, although there are many exceptions; far more frequently the leaves are mixtures of all three colors. The oldest leaves often assume autumnal tints first.

The petiole in transverse section (21) has in form nearly a semi-circle for its dorsal side and a small concave arc as a ventral surface. Under the epidermis lie two or three layers of collenchyma cells. The vascular bundles, of which there are more than 18, are arranged in a flattened circle parallel to the outer surface of the petiole. The pith consists of large, thin-walled cells with very small triangular intercellular spaces. The vascular bundles are separated from each other by broad medullary rays. Large resin ducts are found in the phloem. The primary cortex is bordered internally by a starch sheath. The cells of the xylem have thick and lignified walls. The pith is enclosed by bast fibers and xylem and takes up the largest part of the section. There are no resin ducts in the pith or in the primary cortex.

The leaf in transverse section exhibits palisade parenchyma occupying about one third of the entire thickness of the mesophyl (Pl. III, fig. 4). The spongy parenchyma occupies about five layers of cells. Cells with crystal clusters, presumably of calcium oxalate, occur in the palisade parenchyma. The cells of the lower epidermis are similar to those of the upper epidermis but smaller; stomata are very frequent and apparently absent from the ridges. The leaves wilt very easily. It is hardly possible to bring a cut branch from the field to the laboratory without observing wilting. There are two kinds of trichomes on the leaves, multicellular club-shaped, and unicellular or multicellular bristle-shaped (21).

The thick-walled bristle hairs occur mainly on the lower side on the ridges, large and small, of the leaf, although they are found also in fewer numbers on the upper side in corresponding places. The club-shaped trichomes, on the other hand, are found mostly between the ridges of the leaves. These two different forms of trichomes are similar to those found by Möbius (22) on *Rhus vernicifera* L. and by Rost and Gilg (24) on *Rhus Toxicodendron* L. Morphologically the club-shaped hairs seem to be glandular: first, because the upper multicellular portion is sharply marked off from the basal portion, which resembles a stalk; second, the upper portion has thinner walls than the basal portion; third, they are found mostly on the young, rapidly growing organs of the plant, especially the floral region and the leaves, less on the green stem, and hardly at all on the woody portion. Schwalbe (25, 26) considered the poison of *Rhus diversiloba* to be excreted from glandular hairs on the surface of the plant. That such is not the case can be shown by the two following experiments:

(1) When the green stem, pedicel, or main ribs of the leaf, which are covered with trichomes, are rubbed on sensitive skin, no dermatitis results. Care must be taken, however, that the epidermis of the plant is not broken severely enough to cause the resinous sap to exude.

(2) The fresh green leaves were placed in a finger bowl and soaked at room temperature in 95 percent alcohol for 10 minutes. The leaves had been examined first under a hand lens to make sure that through possible injury no resinous sap was on the surface. When placed in the finger bowl the sap was prevented from running down the pedicel from the cut end into the alcohol. The leaves when taken out of the alcohol had lost their gloss. The pale yellowish alcoholic solution remaining was concentrated by boiling in an open beaker. It was found to be non-toxic. It was not darkened by potassium hydroxide, nor did it respond to other chemical tests for the poison. These results indicate that neither the plant trichomes nor their exudate is poisonous.

The club-shaped hairs are so minute as to be hardly discernible by the naked eye. They have a length of 0.071 mm. and a maximum breadth of 0.0027 mm. Under the microscope they exhibit a clear, unicellular basal portion as an outgrowth of an epidermal cell, above which are the numerous cells that go to make up the main portion of the hair. The cells of the main portion when viewed transversely radiate from a longitudinal central axis. The apex terminates in a single cell, and the entire main portion of the hair is enclosed in a thin-walled sac. The hairs appear to be of two types, which apparently correspond to different stages in development: a densely granular and a sparsely granular form. This difference in granular density is interesting. In animal glands it has long been noticed that when a serous gland has been quiescent for several hours the secretory cells are granular throughout, and the outlines of the cells are only faintly marked as clear lines bounding the granular areas. When the gland secretes, many of the granules disappear and after prolonged secretion very few granules are left; *i.e.*, during secretion the granules normally contained by the cells are in some way or other used up, probably to form a part of the secretion. Although the diminution of zymogen granules is a normal occurrence in the secretion of the salivary, infra-orbital, lachrymal, mucous, and pancreatic glands, yet in the case of the mammary glands the opposite is true, *viz.*, that granules begin to form with the commencement of secretion and do not occur during rest. In the mammary gland, the active growth of protoplasm, the formation of granules from the protoplasm, and the discharge of these granules in the secretion appear to go on at one and the same time. Investigation of the club-shaped hairs of *Rhus diversiloba* has not as yet revealed a positively glandular nature, and consequently a relation between differences in their granulation can not be definitely connected with secretion. From a morphological standpoint, however, as above pointed out, the club-shaped hairs seem to be glandular.

Club-shaped hairs from leaves gathered in the morning before sunrise and from those secured in the heat of the day could not be differentiated. Hairs from rapidly growing leaves could not be distinguished from those of old leaves or stems. Hairs from leaves grown in sunny exposures exhibited no differences, although they were present in greater number than on leaves continuously in the shade.

The Stem

A transverse section of a green stem of *Rhus diversiloba* shows, beginning at the outside, the following tissues (Pl. III, fig. 5): epidermis, with its trichomes and stomata; collenchyma; cortical parenchyma; pericycle, with bast fibers and thin-walled pericycle parenchyma; phloem, with resin ducts; cambium; xylem; medullary ray; pith.

As the stem increases in diameter (fig. 3) the cortex develops a phellogen. The continuous activity of the phellogen results in an increasing thickness of the sheet of cork. The chloroplast-containing tissue beneath the cork layer maintains connection with the air by means of lenticels which have replaced the stomata. As may be anticipated, the dead cork cells are non-poisonous, *i.e.*, they do not cause dermatitis when rubbed on the skin of a susceptible individual and therefore do not constitute a means of transference for the poison.

No resin ducts have been found in the pith of this plant. Engler (5), studying *Rhus Toxicodendron* L., and Inui (10), studying *R. Toxicodendron* var. *radicans*, were unable to discover resin ducts in the pith. Jadin (11) cited 18 species of the genus which are provided with permanent pith resin ducts, and 9 species which do not have them.

At the periphery of the pith the small outer cells acquire a thick wall and become sclerenchymatous. These thick-walled cells may assist the inner large-celled and the outer small-celled pith to maintain a circular outline. A semi-circular row of bast fibers lies external to the primary phloem and serves mechanically to protect the phloem with its resin ducts from external injury.

In the phloem of the second year, new resin ducts appear. These lie neither in radial nor in tangential rows, but are so arranged as to be very nearly equidistant. The first appear in the secondary phloem between two primary resin ducts, and more are formed in a corresponding manner. It must not be forgotten, however, that the formation of resin ducts does not occur in a regular manner.

New bast fibers do not appear to be formed in the pericycle. The epidermis has been almost wholly lost in the second year and is replaced by cork.

The histology of the pith, wood, and bark of the older stems will be treated individually.

The pith cells are polygonal and lie close together; they are generally wider than high, so that their vertical measurement is the smallest. In the specimens examined, the pith cells contained for the most part no particular substance; starch was found sparingly, and tannin sacs appeared as narrow, elongated cells. Tannin sacs, according to Engler (5), appear abundantly in the pith of the Anacardiaceae and in all species of Rhus which he investigated. Pith tannin sacs are not necessarily characteristic of toxic species of Rhus, as Möbius (22) was unable to find them in *R. vernicifera* L.

The bulk of the wood consists of simple pitted wood fibers. In transverse section they are bordered at right angles, and are assembled in rows. The narrower and thicker-walled cells of the fall wood contain starch; the wider and thinner-walled ones of spring wood appear empty.

The pits of the tracheal vessels are exclusively simple with circular or elliptical outlines. The walls are relatively thick. The structure of the vessel wall, where it is in contact with wood parenchyma, is characteristic. In these places simple pits of large size are found chiefly on the vessel wall, and, side by side with them, either transitional or true bordered pits, but no separate bordered pits were noticed. The elliptical pits are transverse to the longitudinal axis of the vessel and parallel to one another, so that they remind one of scalariform perforations.

The medullary rays are, as a rule, uniseriate; sometimes, however, they are biseriate. In tangential longitudinal section they are from three to eighteen cells high; radially their cells are joined together as are the stones in a wall of plane ashlar masonry. The walls of their cells are only moderately thickened, and their lumina are often filled with starch. The medullary rays are most noticeable in the lower part of the stem and in the roots. One small root had five primary medullary rays.

The difference between fall and spring wood rests partly on the dissimilarity of the wood fiber cells and partly on that of the vessels. The first tracheals of spring are larger, thicker-walled, and stretched somewhat radially, while those toward the outer border of the annual ring are flattened to smaller, thicker-walled, and radial rings. The vessels in the spring wood are wider and more numerous, in the fall wood narrower and scarcer, as shown in Plate III, figure 3. The breadth of the annual rings varies.

The inner wood is colored yellow or yellow-brown. A great deal of this coloring matter can be extracted with hot alcohol. This extract behaves similarly to the extract of the related species *Rhus Cotinus* L. (*Cotinus coggygria* Scop.) in the following treatment: an orange-yellow solution in water was made bright yellow by hydrochloric acid, yellow-red by ammonia, orange N. with alum and sodium carbonate solution, and brown N. by calcium chloride solution. Such a behavior by no means proves that the solutes from the wood of *Rhus diversiloba* are identical with those from *R. vernicifera*, although such may actually be the case. The coloring matter is naturally attached to the membrane of the wood cells, which appear golden yellow under the microscope and assume a brown color with caustic potash. Besides the yellow crystals, the wood cavities contain a reddish amorphous resinous substance which is likewise soluble in 95 percent alcohol.

The primary cortex contains sclerosed parenchyma.

The structure of the pericycle is characteristic. It contains many bast fibers, which, in transverse section, have the form of arcs whose convex sides are on the exterior and whose inner concave surfaces surround in each case a single, usually large resin duct (Pl. III, fig. 5).

The resin canals in the later-formed portions of the bark have a lumen and are arranged more or less regularly in concentric circles as heretofore described. The old resin canals appear to be obliterated through a kind of tylotic growth. On one transverse section through the bark of an old stem which has already thrown off the primary covering there are many resin canals differing in form, outline, and dimensions. The innermost are open and nearly circular, but usually more strictly oval in shape, stretched tangentially, and of larger circumference than the outer ones. The outermost, particularly in old stem parts, are entirely or almost entirely obliterated through the luxuriant growth of intruding contiguous tissue. It is possible to observe at different heights of the same resin canal different states of development so that in one place it may still be open and in another closed. This occurrence of tyloses in the secretory ducts is similar to that described by Möbius (22) in *Rhus vernicifera* L., by Leblois in *Brucea ferruginea*, and by Conwentz in the intercellular canals of other plants.

The secondary medullary rays, as already noted, are usually constituted of one row of cells. Where biseriate rays are found, it is sometimes noticed that they split apart tangentially while they remain intact radially. From this it would seem that adjacent cells of the two columns of the medullary ray are only loosely united, whereas those cells which constitute a radial row are more firmly attached.

Besides what has already been said regarding the phloem, it should be added that the sieve tubes and their companion cells extend tangentially and build approximately alternating bands with the layers of phloem parenchyma cells, as in the stem section of *Aristolochia Sipho* (27). The phloem apparently has but little starch, which is found deposited chiefly in the medullary rays. These cells also give a distinct reaction for tannin with ferric chloride.

Morphology and Anatomy of the Root

The root system in its ramifications resembles the crown, in that comparatively few strong branch roots are formed which carry the fine, interlaced roots. The spread of roots depends largely upon the nature of the soil, and upon the supply of food and water. There is a strong tendency to form long lateral roots, particularly in shallow soil. Propagation by layering is very frequently made use of naturally by the plant to insure its food supply and reproduction. The fine, interlacing rootlets are dark brown in color and are covered with fine root hairs of a lighter color. The apical tips of the rootlets are light yellow or colorless for several millimeters.

As in other roots, after the secondary phloem is formed the cambium soon takes on a circular form in section, and behaves in the formation of xylem and phloem exactly as in the stem (Pl. III, fig. 2).

The wood of the root is less firm than that of the stem; there exist numerous large bundles, the fiber cells are less strongly thickened, the medullary rays are broader, being indeed commonly composed of two layers of cells.

Morphology and Anatomy of the Flowers

Rhus diversiloba is strictly dioecious, so far as my observations go. The male and female plants begin to bloom at about the same time. At Berkeley, California, but few of the flowers were open April 4, 1915. The next spring the plants near the Greek Theater at Berkeley bloomed mostly between March 22 and May 1. In 1917 at Pasadena I noticed some male plants at the foot of the Mt. Wilson trail in bloom on the fifth of January. February 28, 1917, the plants of both sexes were just starting to bloom in the Arroyo Seco, south of the Colorado Street bridge, Pasadena. In spite of their yellow-green color, the flower panicles are conspicuously displayed as a result of their size and their accumulation on the ends of the twigs. The presence of the staminate flowers is made very noticeable by their fragrant jasmine or hyacinth aroma. The pistillate flowers, on the other hand, have no apparent perfume. At this point it may be well to mention that an aromatic perfume so similar as to be perhaps identical is noticed when the fresh end of a freshly broken branch is smelled, and that this perfume, unlike that of the flowers, is not confined to the male plant, but is observed also in the female. The similarity between the perfume of the sap and that of the flower becomes more marked upon purification. The "aqueous solution" as made and described in a previous paper (18) contains this more purified sap perfume. The panicles of the male and female flowers are somewhat differentiated as to location and structure.

The flowering shoots of the male plant commonly bear as many flower panicles as leaves, in which case neither the highest leaves nor the lowest leaf develop any panicles in their axils. The lowest leaves of the flowering shoot soon fall off and more readily expose the flower panicles to insects, while the highest leaves remain and tend to protect the blossoms from the direct sunlight, wind, and rain. The panicles are 7 cm. long and stand somewhat stiffly upright at a sharp angle to the axil of the attached twig. The longer ones bear about a dozen side twigs of the first order, of which the three lowest ones are about 2 cm. long and in their turn are again richly branched. Toward the tip the side twigs of the first order become shorter and are not further branched. They are formed like a bunch of grapes, and the end of a panicle is likewise visibly terminated by a flower. The same regularity, as nearly as could be determined, appears in the arrangement of the side twigs of the first order on the panicle stem as was noticed in the phyllotaxy. Minute woolly hairs appear on the panicles at the blooming time, particularly on the bases of the panicle stem and on those of the side twigs.

The flowers are placed singly on stalks from 4 to 7 mm. long, and have a diameter of from 5 to 7 mm. when fully opened. The flowers have 5 calyx leaves, 5 petals, 5 stamens, and one rudimentary ovule; only by way of exception do 6 or 8 stamens occur, and in one flower with 6 stamens 6 petals occurred also.

The calyx leaves are tongue-shaped and have broad bases. They are about 2 mm. long and have a dark green color.

The petals are long-elliptical in shape, narrowed at the base and at the point, and somewhat pointed in the front. They are 4 mm. long and in the middle about 1½ mm. wide. When in bloom the flowers are strongly bent downward. The color of the petals is light green, much lighter than that of the calyx leaves.

The stamens are 2½ mm. long. The white filaments, which are nearly twice as long as the anthers, shove themselves between the anther halves, which somewhat retreat from each other underneath. The anthers are introrse and are borne on upright but slightly curved filaments.

The rudimentary ovary forms a keg-shaped pivot about 1 mm. high, and has 3 discernible stigmas. Between the ovary and the anthers is a disk, which during flowering time glistens with nectar.

Smithsonian Institution
MAY - 2 1921
National Museum

The flower, as viewed from above, is divided into 5 broad lobes, which stand in front of the petals and are separated by the insertion of the stamens; each lobe is again slightly indented in the middle. The outer and inner rims of the disk are somewhat arched toward the top; from this construction a ring-like depression appears in the middle.

While just as many inflorescences as leaves are found on the blossom shoots of the male plants, the number of panicles on the female plant is only about one half as great as that of the leaves. The leaves, however, are more numerous on the blossom shoots of the female. The number of leaves fluctuated between 7 and 9 in several investigations of shoots, while the number of panicles ranged between 3 and 5. As on the male plant, neither the lowest nor the highest leaves bear inflorescences in their axils but only the middle ones. The panicles have a length of 3 to 6 cm. They are not stiffly erect as in the male, but on the contrary only limply placed. The side twigs of the first order are up to 2.5 cm. in length, and have about as numerous branches, but shorter side twigs of the higher order than those of the male. The entire female panicle has about the same general outline as the male panicle. The anatomical structure of the panicle axis is essentially similar to that of the vegetative twig in the first year, and there is no noticeable difference in this respect between the male and the female panicle. Particular structures for tensile strength are not noticed in the axes of the fruit panicles. The stems of the pistillate flowers are not longer than 1 cm. and are often 5 mm. long. The flower itself is smaller than that of the male; its diameter, it is true, measures about 5 mm., but the petals are less curved.

The 5 calyx leaves are somewhat similar to those in the staminate flower, but slightly shorter. The 5 petals are spread out flatter and do not have the curled side rims. They are approximately 3 mm. long and 1.5 mm. broad. Five stamens also occur in the pistillate flower; their anthers are of nearly the same length as the fertile ones of the staminate flower, but the filaments are about 1.5 mm. long and therefore much shorter than those of the male. The anthers are shrunken, of a dirty yellow color, with pollen absent, so that the entire pistillate flower and panicles appear darker. As seen from the broad side, the pistil originates in a somewhat compressed, egg-shaped ovary which is extended in a short style. Toward the top the style spreads out into three thick, brownish stigmas which are beset with papilli. The ovary is also to be considered as constituted by three carpels, of which, however, two are rudimentary so that they appear only in the stigmas. Between the stamens and the ovary is the disk, which is similar to that of the staminate flower except that it is narrower because of the greater expansion of the ovary.

As far as the growth and the finer structure of the flower are concerned the male and female flowers show a great similarity. If one investigates young inflorescences on which the individual flowers are distinguishable as small buds, it is noticed that each flower stands in the axil of a comparatively large carrying leaf which somewhat overhangs the flower. The outside of the bract, as well as the stigma and the axil, are covered with upward-bent trichomes. These trichomes are of two forms, one a single long bristle hair and the other a short, apparently glandular hair with a single-celled base and many-celled ovoid head. These hairs are similar to those previously described as found on the leaves and stems. Further developed flowers, which, with their panicles, are 2 mm. long, have a hairy carrying leaf longer than the panicle. The calyx leaves, the petals, and the stamens lie alongside each other like small enlargements and finally the carpels arise as wall-like growths. In this instance, in which one can clearly recognize the construction of the bud, the stamens are egg-shaped and are covered by the short petals and the longer calyx leaves. Finally the disk shows itself between the gynoecium and the androecium. The course of the vascular bundles may very clearly be recognized in the mounted material, as resin ducts contained in the phloem have their contents turned brown. In the calyx leaf, which is formed with a broad base, 5 ribs appear of which the middle one is the strongest and most branched. On the other hand, the petal, which has a small base, has only one short, weak or unbranched rib on each side of the strongly branched midrib.

The disk appears in longitudinal section as a wide, somewhat sunken cushion. Toward the bottom its tissue is large-celled; above, on the other hand, it consists of small, closely united, plasma-rich cells, of the sort common to glandular tissues. Many small crystal clusters lie on the border of both tissues and in the upper, small-celled tissue, but are lacking in the lower, large-celled tissue. The epidermis consists of rather small polygonal cells and contains numerous stoma-like apertures whose guard cells are almost always larger than the other epidermal cells. A small space is found under the stoma-like opening. These openings apparently do not serve for gaseous interchange, but for the excretion of a glistening and strongly aromatic fragrant nectar whose existence has already been mentioned.

The development of the stamens in pistillate and staminate flowers is apparently similar to the time of the formation of pollen mother cells. In the pistillate flower no pollen grains are formed, the anthers remain empty, and have a shrunken appearance. The filaments of the pistillate flower remain as short as those of the staminate flower until the flowers open. The stamens naturally develop further in the latter. Pollen formation occurs in the anthers but shows nothing particularly noteworthy. The vascular bundles of the anthers contain no resin ducts, these having ended half-way up the filaments. The anther is also to a certain degree the only organ of the plant which has no resin-like or poisonous sap. It is not surprising then that the pollen has no toxic action on the human skin (17). Similar observations have been made by Inui (10) on the pollen of *Rhus vernicifera*, by Warren (29) on that of *R. Vernix*, and by Rost and Gilg (24) on that of *R. Toxicodendron*. The pollen sacs of *R. diversiloba* are composed of two coalesced sporangia, as is common in angiosperms. Their dehiscence occurs by a longitudinal slit, developed where the two coalesced sporangia join. According to Edgeworth (4), the pollen of the Anacardiaceae is oval with 3 slits. The fresh pollen grains of *Rhus diversiloba* are ellipsoidal, about 1/800 sq. mm. in horizontal area, with a width 1/3 to 1/2 the length. The exine is roughened by minute, sharply pointed projections. When the pollen grains are immersed in N/4 KOH they assume a spherical form with no color change. In the material treated (which had been fixed in alcohol and xylol, stained, and mounted in balsam like the rest of the plant material), the spores assumed spherical shapes or in some instances became rounded tetrahedrons. As is common in entomophilous plants, the pollen has no surfaces so modified as to permit the wind to take hold of it, of the nature of the bladder-like appendages of the pine pollen, etc. Whereas anemophilous pollen has a dry outer covering to prevent large masses of pollen from adhering to the flower and hindering wind transportation, the entomophilous pollen of *Rhus diversiloba* is surrounded with a sticky substance so as to adhere to the feet and other parts of the insect. In common with other entomophilous flowers, *R. diversiloba* has perfume-secreting glands heretofore described which may serve to attract insects. The pollen itself being non-toxic and not wind-blown, the aerial transmission of the poison by the agency of pollen is quite out of the question.

As in the female flower the stamens develop to a certain advanced stage, so the ovary develops in the male flower to the extent that an almost fully developed ovule is produced. Such development of an ovule in a flower which is functionally purely staminate, borne on a purely male plant, is a phenomenon which has been but rarely observed. Each ovary contains regularly but one ovule. The funiculus becomes curved at its apex, so that the body of the ovule lies against it, and, although the axis of the body is straight, the micropyle is directed towards the surface of origin; thus the funiculus appears as a ridge along one side of the body of the ovule, and the ovule is anatropous and consequently of the form most common among angiosperms.

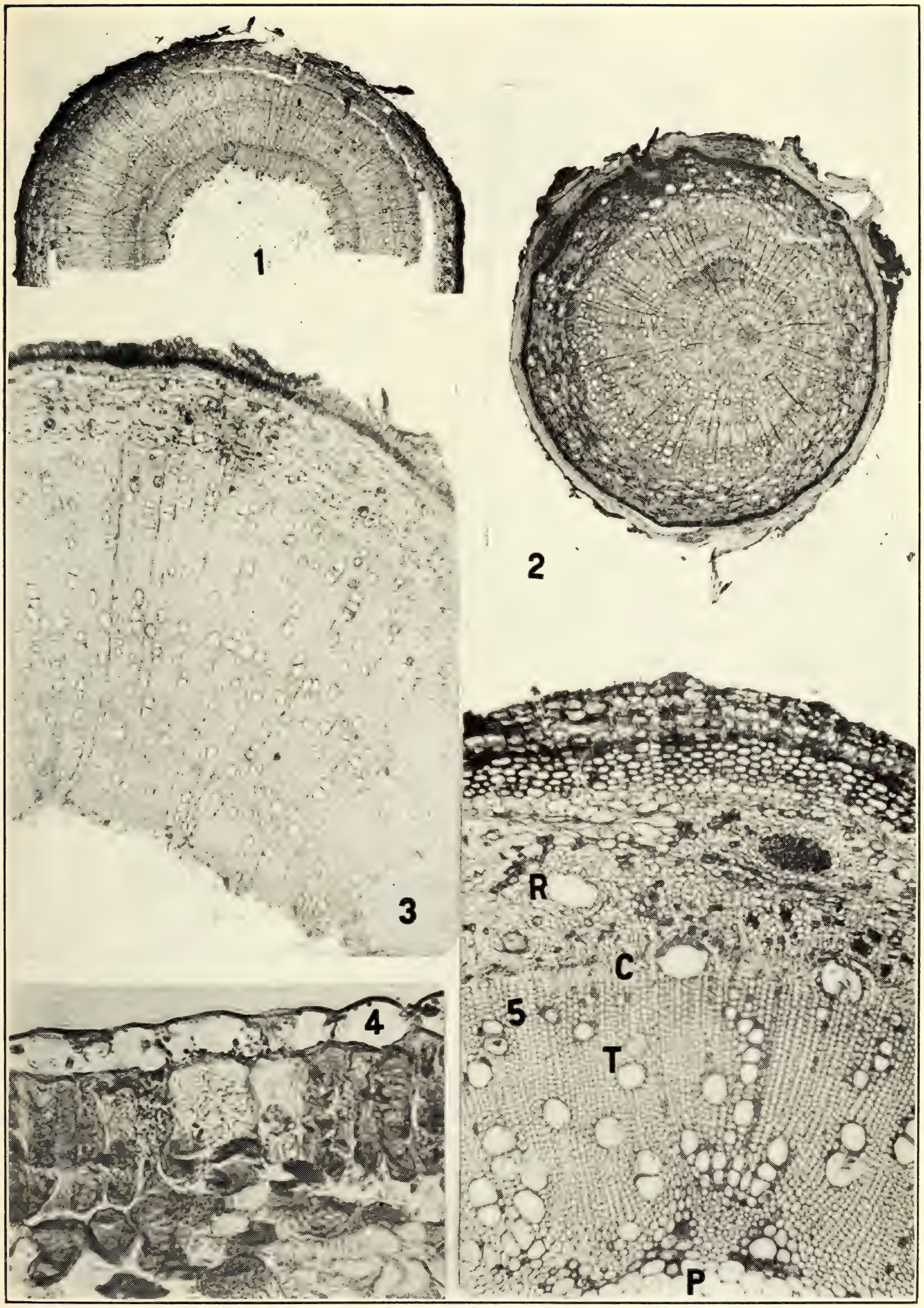
The ovule, in the mature female flower, fills the ovarian cavity. The outer integument, therefore, occupies considerable space. The micropyle is somewhat widely removed from the upper arching of the nucellus. The inner integument is widely tubular and lengthened outwardly over the nucellus, in which the embryo sac is again somewhat pressed back toward the inside so that a wider path is prepared for the pollen tube. The advantage of an anatropous ovule is apparent when it is remembered that the pollen tube advances along the wall of the ovary, and that the micropyle is thus brought near the wall. It is not surprising, then, that this plant with its efficient apparatus for fertilization should have large fruit production.

Numerous germinating pollen grains are found on the stigmas of open pistillate flowers. The pollen tubes grow inside between the stigma papillae and pass through 4 to 6 cells of which the upper one is longest and thickest. On the stigmas of the staminate flowers such papillae are not formed, so that here no pollen grains are found. The wall of the ovary is penetrated by numerous vascular bundles with resin ducts which continue to the upper end of the pistil where the resin ducts terminate blindly with pointed ends.

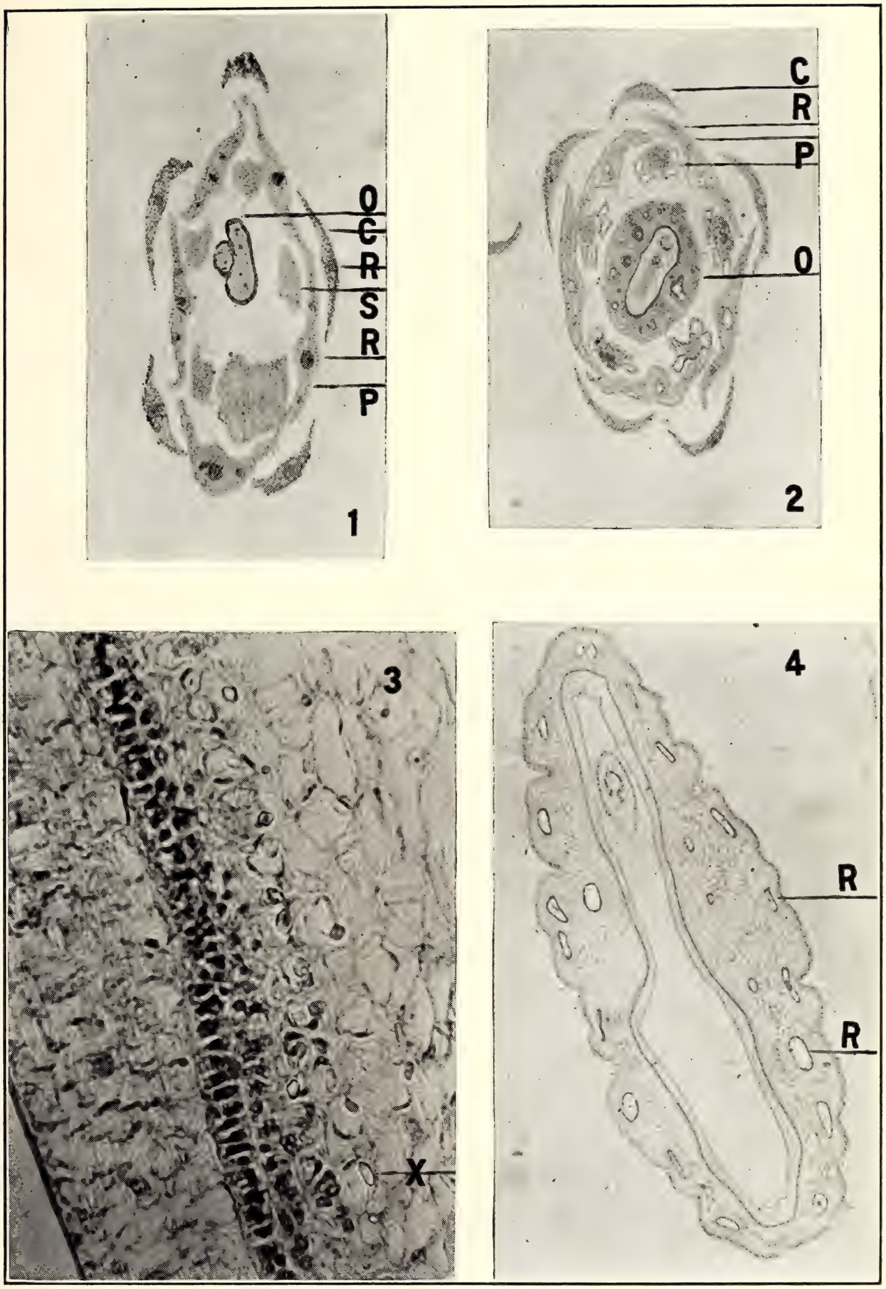
The development of the fruit, which terminates the life of the plant, has been taken up in another paper (19).

LITERATURE CITED

1. **Abrams, L.** Flora of Los Angeles and vicinity. Stanford University, 1911.
2. **Brandegee, T. S.** A collection of plants from Baja California. Proc. Cal. Acad. Sci., ser. 2, **2**: 140. 1889.
3. ——. The plants of Santa Catalina Island. Zoe **1**: 110, 134. 1890.
4. **Edgeworth, M. P.** Pollen. London, 1879.
5. **Engler, A.** Über die morphologischen Verhältnisse und die geographische Verbreitung der Gattung Rhus, wie der mit ihr verwandten lebenden und ausgestorbenen Anacardiaceen. Bot. Jahrb. Syst. Pflanzengesch. u. Pflanzengeographie **1**: 365–426. Taf. 4. 1881.
6. **Hall, H. M.** A Yosemite flora, p. 151. San Francisco, 1912.
7. **Hooker, W. J.** Flora boreali-americana **1**: 127. London, 1833.
8. ——**, and Arnott, G. A. W.** The botany of Captain Beechey's voyage. Part 3, p. 137. London, 1841.
9. **Howell, T.** A flora of northwest America **1**: 119. Portland, Ore., 1898.
10. **Inui, T.** Ueber die Gummiharz-Gang des Lackbaumes und seiner verwandten Arten (Abstr.). Bot. Centralbl. **83**: 352. 1900.
11. **Jadin, F.** Observations sur quelques Térébinthacées. Jour. de Bot. **7**: 382–390. 1893.
12. ——. Origine des sécréteurs. Thése. Montpellier, 1888.
13. **Jepson, W. L.** A flora of western and middle California. 2nd ed., p. 249. Berkeley, 1911.
14. **Leblois, A.** Recherches sur l'origine et le développement des canaux sécréteurs et des poches sécrétrices. Ann. Sci. Nat. Bot. VII, **6**: 247–330. 1887.
15. **Lindley, J.** *Rhus diversiloba.* Various leaved poison oak. Edwards' Bot. Reg. n. ser. **18**: 38. 1845.
16. **Lyon, W. S.** The flora of our southwestern archipelago. II. Bot. Gaz. **11**: 330–336. 1886.
17. **McNair, J. B.** The transmission of Rhus poison from plant to person. *Rhus diversiloba* T. and G. Jour. Infect. Dis. **19**: 429–432. 1916.
18. ——. The poisonous principle of poison oak. Jour. Amer. Chem. Soc. **38**: 1417–1421. 1916.
19. ——. Fats from *Rhus laurina* and *Rhus diversiloba.* Bot. Gaz. **64**: 330–336. 1917.
20. ——. The oxidase of *Rhus diversiloba.* Jour. Infect. Dis. **20**: 485–498. 1917.
21. ——. A study of *Rhus diversiloba* with special reference to its toxicity. Amer. Jour. Bot. **8**: 127–146. 1921.
22. **Möbius, M. A.** Der Japanische Lackbaum. Abhandl. Senckenberg. Naturforsch. Ges. **20**: 201–247. 1899.
23. **Piper, C. V.** Flora of the state of Washington. Contrib. U. S. Nat. Herb. **9**: 384. 1906.
24. **Rost, E., and Gilg, E.** Der Giftsumach, *Rhus Toxicodendron* L., und seine Giftwirkungen. Ber Deutsch. Pharm. Ges. **22**: 296–358. 1912.



McNAIR: MORPHOLOGY OF RHUS.


O
C
R
S
R
P
1
C
R
P
O
2
3
X
4
R
R

McNair: Morphology of Rhus.

25. **Schwalbe, C.** On the active principle of *Rhus diversiloba*. Med. Rec. **63**: 855. 1903.

26. **Schwalbe, K.** Die giftigen Arten der Familie Rhus. Münch. Med. Wochenschr. **49**: 1616. 1902.

27. **Strasburger, E., and others.** A text book of botany. Eng. transl. by H. C. Porter. p. 122. London, 1898.

28. **Torrey, J., and Gray, A.** A flora of North America **1**: 218. New York, 1838.

29. **Warren, L. E.** Some observations on the pollen of poison sumach. Amer. Jour. Pharm. **85**: 545–549. 1913.

EXPLANATION OF PLATES

PLATE III

All figures have been reduced one half in reproduction and now show magnifications as follows: figure 1, ×10; figure 2, ×10; figure 3, ×23.3; figure 4, ×470; figure 5, ×91.65.

FIG. 1. Transverse section through the same stem as in figure 3.

FIG. 2. Transverse section through a woody root.

FIG. 3. Transverse section through a stem older than that of figure 5, showing annual rings with their varied formations of spring and fall growth.

FIG. 4. Transverse section through mature leaf showing cystolith in palisade parenchyma.

FIG. 5. Transverse section through stem showing cork cambium; tracheal tube (*T*); pericycle with schlerenchyma cells or bast fibers and thin-walled pericycle parenchyma; phloem with resin duct (*R*); cambium (*C*); pith (*P*).

PLATE IV

All figures have been reduced one half in reproduction and now show magnifications as follows: figure 1, ×23.3; figure 2, ×23.3; figure 3, ×470; figure 4, ×23.3.

FIG. 1. Transverse section through a male flower near its base, showing 5 calyx leaves (*C*) with resin ducts (*R*), 5 petals (*P*) with resin ducts (*R*), 5 stamens (*S*), and the nonfertile ovule (*O*).

FIG. 2. Transverse section through a female flower near its apex, showing 5 calyx leaves (*C*) with resin ducts (*R*), 5 petals (*P*) with resin ducts, 5 rudimentary anthers with neither pollen nor resin ducts, and the fertile ovule (*O*).

FIG. 3. Transverse section through an unripe fruit near the seed, showing numerous crystals. Size of hexagonal crystal, 0.007 × 0.0025 mm.

FIG. 4. Transverse section through an unripe fruit showing an abundance of resin ducts (*RR*). Diameter of largest resin duct, 0.0085 mm.

DISTRIBUTION OF THE MALVACEAE IN SOUTHERN AND WESTERN TEXAS[1]

Herbert C. Hanson

(Received for publication December 6, 1920)

Introduction

In connection with the eradication of the pink bollworm of cotton the writer was assigned the project of studying the distribution and abundance of the malvaceous plants in various parts of Texas. This work was done under the direction of Dr. W. D. Hunter, in charge of the pink-bollworm eradication work of the Federal Horticultural Board. The reason for making this survey was to determine if the malvaceous plants other than cotton were of importance in relation to the eradication of the pink bollworm (*Pectinophora gossypiella* Saunders). Throughout the entire survey no indication of the insect was found on any of the malvaceous plants other than cotton.

From June to December, 1918, and in June and November, 1919, the extreme southeastern section of the state, embracing Hardin, Jefferson, Liberty, Chambers, Galveston, and parts of Harris, Fort Bend, and Brazoria counties, were thoroughly scouted. In June, 1919, the vicinity of Hearne, 100 miles northwest of Houston, was examined. In June and July, 1919, the areas in the vicinity of Corpus Christi, San Antonio, and Pecos were studied. From January to June, 1919, and in August, 1919, a strip 20 to 80 miles wide on the Texas side of the Rio Grande from the Gulf of Mexico to New Mexico was scouted.

The species of Malvaceae found in the areas studied are discussed under the following life zones: 1. Semi-tropical Gulf Strip of the Lower Austral Zone; 2. Austroriparian Division of the Lower Austral Zone; 3. Lower Sonoran Division of the Lower Austral Zone; and 4. Upper Sonoran Division of the Upper Austral Zone.

I. Semi-tropical Gulf Strip of the Lower Austral

The Semi-tropical Gulf Strip includes that part of Texas along the coast below the 100-foot contour line. This strip, involving approximately the Coast Prairie, is about 60 miles wide. It is divided into the Humid Section, the area east of the 97th meridian; and the Xerophytic Section, the area west of the 97th meridian.

---

[1] Published with the permission of the Secretary of Agriculture.

A. Humid Section

Topography: Usually low and flat with occasional low hills. Drainage is poor, so there is much marshy land. Adjacent to the coast is a strip of salt marsh, often several miles wide. *Soil:* Black clay chiefly. *Elevation:* Most of the area is below 100 feet; Beaumont 29 feet, Galveston 9 feet. *Rainfall:* The annual mean in the eastern part is over 50 inches and in the western part about 35 inches; at Beaumont, 45.1 inches; at Galveston, 47.6 inches. *Temperature:* The mean annual temperature at Beaumont is 68° F., at Galveston 69.4° F. *Clear Days:* The percentage of clear days in 1918 at Beaumont was 38.8, at Galveston 28.8. *Humidity:* Very high, as is indicated by the abundance of Spanish moss (*Tillandsia* spp.) on trees.

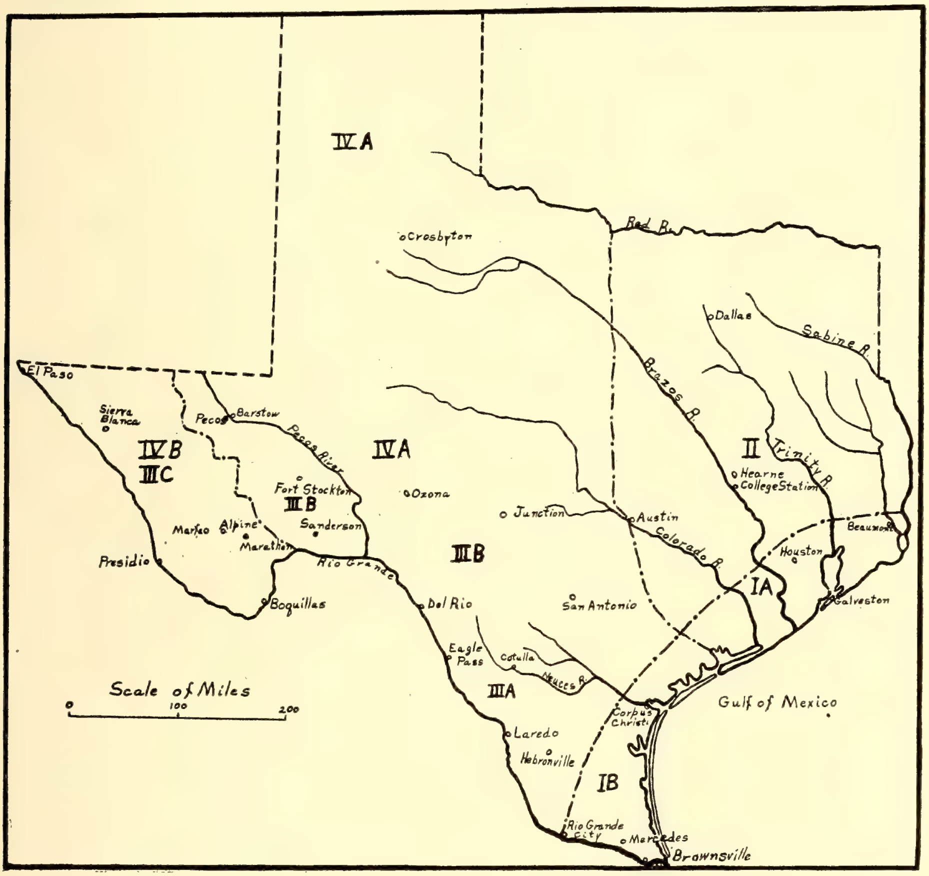

IVA
Red R.
Crosbyton
Dallas
Sabine R.
Brazos R.
II
Trinity R.
Hearne
College Station
Beaumont
Houston
IA
Galveston
Austin
Colorado R.
Junction
Ozona
IVA
El Paso
Sierra Blanca
Pecos
Barstow
Pecos River
IVB
IIIC
Fort Stockton
IIIB
Marfa
Alpine
Marathon
Sanderson
Presidio
Rio Grande
Boquillas
Del Rio
IIIB
San Antonio
Eagle Pass
Cotulla
Nueces R.
IIIA
Scale of Miles
0
100
200
Gulf of Mexico
Corpus Christi
Laredo
Hebronville
IB
Rio Grande City
Mercedes
Brownsville

FIG. 1. Map of Texas, showing life-zones. I. Semi-tropical Gulf Strip: *A*. Humid Section, *B*. Xerophytic Section. II. Austroriparian. III. Lower Sonoran: *A*. Rio Grande Plain, *B*. Great Plains, *C*. Plateaus and mountains below 4000 feet. IV. Upper Sonoran: *A*. Great Plains, *B*. Plateaus and mountains above 4000 feet.

Most of this section is open prairie characterized by such genera as Rudbeckia, Oenothera, Sabbatia, Panicum, Baptisia, Eragrostis, Andropogon, and others. From the east the Atlantic type of woodland is invading.

The most common and abundant species is *Pinus taeda*. Other common species are: *Quercus stellata*, *Q. michauxii*, *Pinus palustris*, *Taxodium distichum*, *Hicoria* spp., *Ulmus* spp., *Magnolia* spp., *Liquidambar styraciflua*, *Nyssa* spp., *Ilex* spp. From the southwest, *Parkinsonia aculeata*, *Prosopsis glandulosa*, and *Vachellia Farnesiana* are invading. Characteristic of the salt marshes are species of Spartina and Sporobolus.

Nine wild malvaceous species were found in this area. The only species found on the prairie was *Callirrhoe involucrata*. In the fresh water swamps *Hibiscus lasiocarpus* was common and abundant, *H. militaris* and *Kosteletzkya althaeifolia* were infrequent. In the brackish marsh at the mouth of the Trinity River in Galveston Bay, *Kosteletzkya althaeifolia* grew very vigorously and abundantly, *Hibiscus militaris* and *H. lasiocarpus* rarely. *Malvaviscus drummondii* occurred commonly and often abundantly in fairly open woods along streams, lakes, bays, and in shrubby thickets on the edges of woods. *Sida spinosa* and *Malvastrum americanum* were found rarely in very open woods. No malvaceous plants were found in the pine or swamp forests. Four malvaceous weeds were found: *Sida rhombifolia* abundant and common in waste places, *S. spinosa* frequent in fields, *Malvastrum americanum* frequent in waste places, and *Modiola caroliniana* rare in low, moist, waste places.

B. Xerophytic Section

Topography: Low and flat, broken only by streams and very low hills and ridges. In the northern part of Cameron County an extensive area of sand dunes extends inland from the Gulf. *Soil:* Chiefly compact clay. *Elevation:* Most of the area is below 100 feet; Corpus Christi is 20 feet, Brownsville 38 feet, Mercedes 68 feet. *Rainfall:* The annual mean at the 97th meridian is about 35 inches, at the Rio Grande about 20 inches. The annual mean at Corpus Christi is 27 inches, at Brownsville 27 inches (maximum 60 inches, minimum 9 inches), at Mercedes 19.8 inches. *Temperature:* The mean annual temperature from Laredo to the Gulf is 73° F., at Brownsville 73° F., at Corpus Christi 70.0° F. *Clear Days:* The percentage of clear days in 1918 in Corpus Christi was 36, in Mercedes 12.1. *Humidity:* The amount of water evaporated from a free-water surface, according to Hill (9), is less than 60 inches annually.

Most of the Xerophytic Section is covered with chaparral vegetation. Woody species of the Leguminosae; mesquite (*Prosopsis glandulosa*), huisache (*Vachellia Farnesiana*), mimosas, acacias, *Parkinsonia aculeata*, prickly pear (*Opuntia* spp.), *Leucophyllum texanum*, and others characterize this vegetation. Along streams and lakes, *Ehretia elliptica*, *Celtis pallida*, *Siderocarpus flexicaulis*, and large mesquites and huisaches are common. Dense clumps of the Mexican palm, *Sabal mexicana*, occur along the Rio Grande in the vicinity of Brownsville. A greater abundance of tropical vegetation is prevented from developing by the periodic freezes caused by "northers" in winter and by the aridity of the region.

Several of the malvaceous species found in this area were very rare, and some had not been previously collected in the United States. *Abutilon incanum* was found commonly in the taller chaparral, in open woods, and as a weed in fields throughout the region. *A. berlandieri* occurred in the same habitats as *A. incanum*, but it was less abundant. *A. jacquini* was found only in small clumps, or scattered singly, in openings in the palm woods between Brownsville and the Gulf. Only one clump, composed of about 20 plants, of *A. pedunculare* was found, in a small opening in the palm woods below Brownsville. *A. triquetrum* formed large clumps in openings in the palm woods and was found rarely in mesquite and huisache woods within a few miles of Brownsville. A few plants of *A. wrightii* were found on the shell point extending into the bay between Portland and Corpus Christi. *Bastardia viscosa* was found in very limited numbers in only one locality, in prickly pear and mesquite on the shore of a lake east of Brownsville. *Callirrhoe digitata* occurred abundantly in low thickets in the vicinity of Corpus Christi. *Cienfuegosia sulphurea* was found commonly on low, moist, black clay soil in the vicinity of Corpus Christi, Taft, and Gregory. It appears to have spread considerably since Heller (8) found it at Corpus Christi in 1894, and Lewton (11) found it at Taft in 1910 after considerable searching. The thick, woody tap root of the plant makes it a persistent weed in low parts of cotton fields. *Hibiscus cardiophyllus* was found rarely in mesquite woods near Brownsville, rarely on the dry clay banks of the Arroyo Colorado near Harlingen, and rather frequently in the chaparral west of Harlingen. In places it is cultivated for its pretty red flower. *Malachra capitata* was found only in the Brownsville region, occurring rarely in openings in the palm woods, on the borders of the willow woods along the Rio Grande, and as a weed in fields. *Malva parvifolia* occurred very commonly and abundantly as a weed in towns of this section. *Malvastrum americanum* was found commonly in woods and as a weed in fields and yards. *M. spicatum* was found frequently in open woods, on the edges of woods, and as a weed from Brownsville to Mission. In very open woods of mesquite, huisache, and Texas ebony along a lake near Brownsville, *M. americanum* and *M. spicatum* were the dominant plants under and between the trees. *M. wrightii* was common and abundant in open mesquite and as a weed along fences and roads in the vicinity of Corpus Christi. *Malvaviscus drummondii* was very abundant and common in the palm woods and in thickets along the Rio Grande and in the woods near Corpus Christi. It is often cultivated for its odd red flower and red berry-like fruit. *Modiola caroliniana* was found very rarely in low, moist, open places near Brownsville. *Sida angustifolia* and *S. spinosa* were found infrequently as weeds in fields and in open woods. *S. ciliaris* was found in open woods and as a weed in fields and lawns from Riviera to Gregory. *S. filiformis* and *S. diffusa* were found frequently in open mesquite woods and in openings from Brownsville westward. *S. hastata* occurred commonly as a weed in towns and in open chaparral from Brownsville westward. *S. cuneifolia* was found abundantly in this region on sandy ridges near Point Isabel. One plant of a form of *Sphaeralcea lobata* was found in a vacant yard near Brownsville. No other specimens of this western species were found east of the Pecos River. *Wissadula lozani* was common in open woods and as a weed along roads in the vicinity of Corpus Christi and from Brownsville westward. *W. periplocifolia* was found abundantly in openings and along the edges of palm woods, and frequently in other woods in the vicinity of Brownsville only.

II. Austroriparian Division of the Lower Austral

Area: The part of Texas east of the 98th meridian and north of the Semi-tropical Gulf Strip. *Topography:* Chiefly prairie, low rolling hills in places. *Soil:* The soil varies considerably from sand to clay. There are large areas composed of heavy, black clay soil. *Elevation:* From about 100 feet in the southern part to about 1000 feet in the northwestern part; at College Station 308 feet, at Dallas 512 feet. *Rainfall:* The annual mean in the eastern part is over 50 inches, in the western part about 35 inches; the annual mean at College Station is 37.5 inches, at Dallas 38.0 inches. *Temperature:* The mean annual temperature at College Station is 67.9° F., at Dallas 66.4° F. *Clear Days:* The percentage of clear days in 1918 at College Station was 34.3, at Dallas 44.7. *Humidity:* The evaporation from a free-water surface, according to Hill, is less than 50 inches annually for the division.

As only one locality, the vicinity of Hearne in the southern part of this section, was scouted, no attempt is made at listing all the malvaceous species that occur in the entire section. The low, red, sandy clay hills in the vicinity of Hearne are covered with oak forest, and the wide river bottom of the Little Brazos River, consisting of black clay loam, supports a forest of hickories, oaks, magnolias, elms, sycamores, and others.

Malvaviscus drummondii was found commonly, but not abundantly, in the bottom-land forest and in thickets on the borders of this forest. *Callirrhoe involucrata* occurred abundantly and commonly in woods, in waste places, along roads, and in clearings in this locality. *Sida diffusa* was very rare as a weed in waste places. *Modiola caroliniana* occurred very commonly and abundantly as a weed in low, moist waste places. The largest and best developed *Modiola* plants found in Texas were in Hearne.

III. Lower Sonoran Division of the Lower Austral

A. The Rio Grande Plain

Area: The Rio Grande Plain comprises the area bounded by the Gulf Coast Strip, by the Rio Grande, and by a line drawn from Del Rio to San Antonio to the Gulf Coast Strip near Corpus Christi. It is separated from the Great Plains to the north, between San Antonio and Del Rio, by the Balcones Fault Escarpment. The rocks north of this escarpment were lifted up relatively to those south of it. *Topography:* Marked by flat silt plains, sandy plains, low sandy hills, arid clay hills, and ridges of coarse gravel. The surface is more hilly and broken in the western and northern parts than near the Gulf. The Rio Grande flows through a broad, sometimes terraced, valley from Del Rio to the Gulf. *Elevation:* From about 100 feet where the area borders the Gulf Coast Plain to about 1,000 feet at the Balcones Escarpment; at Del Rio 952 feet, at San Antonio 701 feet, at Eagle Pass 800 feet. *Rainfall:* The annual mean ranges from 20 to 25 inches over most of the area; Del Rio 21.0 inches, San Antonio 26.8 inches, Eagle Pass 20.9 inches. *Temperature:* The mean annual temperature at Del Rio is 69.6° F., at San Antonio 70.3° F., at Eagle Pass 71.1° F. and from Laredo to the Gulf 73.0° F. *Clear Days:* The percentage of clear days in 1918 at Del Rio was 57.0, at San Antonio 50.9, at Eagle Pass 50.9. *Humidity:* According to Hill, the mean annual evaporation from Rio Grande City to Eagle Pass is 50–60 inches, from Eagle Pass to Del Rio 60–65 inches.

On the level plains mesquite is dominant. Other important species associated with it are *Condalia obovata*, huisache in the moister areas, *Quercus virginiana*, and others. Grasses and many herbs of tropical affinities characterize the sandy plains in the southeastern part. Great Plains grasses and herbaceous species are common in the northwestern part. On the dry hills and ridges, prominent chaparral plants are *Covillea tridentata*, *Acacia* spp., *Parkinsonia texana*, and *Leucophyllum texanum*. The great variety of cacti and yuccas in the western part of the region indicates a greater degree of xerophytism. Along the Rio Grande hackberries, hickories, elms, sycamores, and huisaches are conspicuous.

Abutilon incanum was found frequently in this region in the woods along the Rio Grande, in mesquite chaparral on the plains, on rocky and gravelly hills, and as a weed along roads. *A. berlandieri* was rare in the woods along the Rio Grande and in mesquite woods along creeks. *A. wrightii* was frequent on rocky hillsides. *Callirrhoe digitata* was abundant in mesquite chaparral in the vicinity of Del Rio and San Antonio. *C. involucrata* occurred frequently in grassy valleys and plains from Laredo west and north. *C. lineariloba* was found only on the sandy, grassy plains in the vicinity of Hebronville. *Gayoides crispum* grew abundantly on exposed rocky slopes, mesquite-covered clay slopes, and stream banks in the vicinity of Laredo and westward. *Hibiscus cardiophyllus* was frequently found in mesquite chaparral on clay hills, on sand and gravel hills, and on rocky cliffs along the Rio Grande as far west as the vicinity of Del Rio. *H. coulteri* was found frequently on rocky hillsides along the Rio Grande in the vicinity of Del Rio. The region about Del Rio appears to be the eastern limit of *H. cardiophyllus*. *Malvastrum americanum* occurred frequently as a weed in towns and fields and in woods in valleys. *Malvaviscus drummondii* was very abundant in the mesquite woods along streams in the vicinity of San Antonio, but rare in the Rio Grande Valley. *Modiola caroliniana* was found infrequently as a weed in low, moist places in the vicinity of San Antonio. *Malva parviflora* was a very common and abundant weed in waste places throughout the region. *Sida cuneifolia* was found commonly in the sandy plain and on sandy hills from Hebronville to

Laredo. *S. angustifolia* occurred frequently in valleys and as a weed in fields from the Gulf Coast Strip to Laredo and Cotulla. *S. diffusa* and *S. filiformis* were common on rocky and gravelly hills, on dry clay hills, on plains, and in valleys throughout the region. *S. hastata* occurred commonly in valleys, on plains, and on rocky and sandy slopes, and as a weed along streets. *Sphaeralcea cuspidata* was found abundantly in sandy, loamy, and clay soil in valleys and as a weed along irrigation ditches and in fields from Eagle Pass westward. *S. hastulata* was abundant on the sandy plain and in gravelly places south of Hebronville. *S. pedatifida* was frequent on sandy and gravelly slopes from Laredo to Cotulla and Eagle Pass.

B. The Great Plains

Area: The Great Plains includes the area from the Balcones Fault Escarpment between Del Rio and San Antonio west to the mountains in Trans-Pecos Texas and northwest throughout Texas. The portions of this area below 4,000 feet belong to the Lower Sonoran; the areas above 4,000 feet belong to the Upper Sonoran. *Topography:* The southern part is dissected into rugged hills and deeply eroded ravines and canyons. The area west of the Pecos River is more elevated and rougher than the area east of it. The northern part is generally quite flat. *Elevation:* 1,000 to over 4,000 feet; at Junction 2,180 feet, at Barstow 2,573 feet, at Fort Stockton 3,050 feet, at Plainview, near Crosbyton, 3,370 feet. *Rainfall:* For most of the area the mean annual precipitation is from 15 to 20 inches. At Junction it is 25.1 inches, at Barstow 11.1 inches, at Fort Stockton 15.1 inches, at Crosbyton 21.0 inches. *Temperature:* The mean annual temperature at Junction was 65.4° for 1918, at Barstow 64.5°, at Fort Stockton 64.0°, at Crosbyton 59.7°. *Clear Days:* The percentage of clear days at Junction in 1918 was 59.0, at Barstow 64.4, at Fort Stockton 44.2, at Crosbyton 59.7. *Humidity:* The mean annual evaporation, according to Hill, ranges from 60 to 80 inches per year throughout the region.

The southern part of this region is a sparsely wooded area, the northern part a short-grass area. Characteristic plants of the southern part are mesquite, *Acacia* spp., *Mimosa* spp., hackberries, pecan, oaks, piñon pine, mountain cedar, Covillea, cacti, yucca, and agaves. The bald cypress (*Taxodium distichum*) finds its western limit in this region.

Abutilon incanum was found frequently in wooded valleys, in rocky ravines, and on rocky slopes from Ozona to Fort Stockton and southward through the area. *Abutilon wrightii* was found frequently on rocky slopes and cliffs in the southern part of the region. *A. texense* was rare in open mesquite in valleys from Ozona to the Pecos River, and in the shade of mesquites in the depressions on the plains near Barstow. *Callirrhoe digitata* was abundant in thickets along the lower part of the Devils River. *C. involucrata* was common and abundant in woods and in openings in valleys from Del Rio to Ozona and Sheffield. *Disella lepidota* was abundant in slight depressions in the plains in the vicinity of Pecos. *Gayoides crispum* was found frequently in rocky situations in the southern part. *Hibiscus cardiophyllus* was rare on rocky banks of the Rio Grande near Del Rio.

H. coulteri occurred frequently on rocky hillsides and cliffs along the Rio Grande. *Malva parviflora* was a common weed in waste places in towns. *Pavonia lasiopetala* was found in only one place, in a dry, rocky ravine near New Braunfels. *Sida diffusa* and *S. filiformis* occurred commonly in sandy and rocky places, in chaparral, and on the open plains from the Rio Grande to Pecos. *S. hastata* was frequent in woods, open plains, in rocky places, and as a weed in waste places throughout the region. *Sphaeralcea cuspidata* was frequent in sandy to clay soil in valleys and on low plains and as a weed in fields from the Rio Grande to Pecos. *S. lobata* was found commonly as a weed along irrigation ditches at Pecos and at Barstow. *S. subhastata* was a common weed adjoining fields near Pecos.

C. Trans-Pecos Plateau and Mountains below 4000 feet

Area: Extends from the Great Plains on the east to New Mexico on the west and from the Rio Grande to New Mexico on the north. This area belongs to three climatic zones: the Lower Sonoran below 4,000 feet, the Upper Sonoran above 4,000 feet, and the Transition on the highest peaks of the Davis and Guadalupe mountains. *Topography:* This is an area of low mountains separated by plains and basins that have been formed mostly from the *dêbris* derived from the erosion of the mountains and by lava flows. Most of the mountains are broad and somewhat flat. *Soil:* In many arid and semi-arid localities "caliche" is a characteristic formation. "Caliche" is a deposit of lime formed by the evaporation of waters carrying calcium carbonate. This forms the "cap rock" of the high plains. *Elevation:* In the Rio Grande Valley near Boquillas the elevation is about 1,500 feet. The highest elevation is 8,690 feet, on El Capitan in the Guadalupe Mountains near the New Mexican line. At Fort Davis the elevation is 5,000 feet, at El Paso 3,762 feet. *Rainfall:* The annual mean for most of the region is from 9 to 20 inches; at Fort Davis it is 17.4 inches, at El Paso 9.8 inches. *Temperature:* The annual mean temperature at El Paso is 62.9°. *Clear Days:* The percentage of clear days at El Paso in 1918 was 56.4. *Humidity:* The mean annual evaporation for this region, according to Hill, is 70–90 inches.

In the Rio Grande Valley, willows, cottonwoods, mesquite, and screwbean (*Strombocarpa pubescens*) are important species. On dry plains and slopes, yuccas, agaves, numerous cacti, *Dasylirion* spp., *Nolina* spp., *Fouquiera splendens, Covillea tridentata,* low mesquites, shrubby acacias, and mimosas are abundant. Open short-grass plains separate the mountains in the vicinity of Alpine and Marfa. On higher slopes, in the Upper Sonoran Zone, *Pinus edulis, Juniperus* spp., and *Quercus* spp. compose the chief elements of the vegetation.

Abutilon incanum and *A. parvulum* were found rarely, *A. wrightii* frequently, on rocky slopes in this area. *A. malacum* was abundant on rocky hillsides and mountain sides along the Rio Grande from Boquillas to El Paso and in the vicinity of Balmorhea. *Hibiscus denudatus* var. *involucellatus* was frequent from Boquillas and Marathon throughout the Big Bend to El Paso on rocky slopes. *H. coulteri* was found in similar situations, but ranged east to the Great Plains. *Malva parviflora* occurred rarely as a weed in towns. *Disella lepidota* was common along the Rio Grande in the Big Bend. *Disella hederacea* was common in somewhat alkaline soil along the Rio Grande at El Paso and at Indio in the Big Bend. *Sida hastata* occurred generally but not abundantly in valleys throughout the region. *S. diffusa* and *S. filiformis* were common in their usual wide range of habitats. *Gayoides crispum* was found frequently on rocky slopes in the Big Bend and in the Davis Mountains. *Sphaeralcea cuspidata* was found abundantly in the Rio Grande Valley from Boquillas to El Paso, and infrequently in the valleys to the north. *S. lobata* was abundant in rocky and sandy soil near El Paso, and rare at Ruidosa in the Big Bend. *S. incana* was rare below 4,000 feet, but it was seen in sandy soil near the Rio Grande at El Paso. *S. subhastata* was rare at the base of mountain slopes in sandy clay soil at Shafter and Sierra Blanca. *S. tenuipes* was found in abundance on rocky mountain sides at El Paso and Sierra Blanca. *Sida longipes* was rare on rocky mountain sides near Sanderson. *S. tragiaefolia* was common in rocky valleys at Boquillas.

IV. The Upper Sonoran

The higher portions of the Great Plains, already discussed under the Lower Sonoran, are included in the Upper Sonoran. As very little of the Upper Sonoran plains were scouted, this area is not treated in detail. The Trans-Pecos Plateau and Mountains above 4,000 feet are included in the Upper Sonoran. The physiography, climate, and vegetation of this area were described in the preceding section.

Hibiscus denudatus var. *involucellatus*, *H. coulteri*, *Abutilon incanum*, *A. malacum*, *A. wrightii*, *A. parvulum*, *Gayoides crispum*, *Sphaeralcea cuspidata*, *S. lobata*, *S. incana*, *Sida hastata*, *S. diffusa*, and *S. filiformis* were found in this zone in locations as described in the preceding section. *Wissadula holosericea* was frequent on rocky slopes near Balmorhea, in the Davis Mountains, and near Alpine. *Sphaeralcea tenuipes* was abundant on the mountain sides near El Paso. *S. pumila* was frequent in sandy soil on lower mountain slopes near the lower limit of this zone at El Paso. *Malvastrum elatum* occurred frequently in sandy and rocky soil from Alpine and the Davis Mountains to El Paso. *M. coccineum* was rare on the sandy plain near Alpine. A few plants of *Disella sagittaefolia* were found as weeds in a yard at Alpine. *Sida tragiaefolia* was rare on rocky slopes near Alpine. *S. longipes* was rare on rocky slopes from Sanderson to Marathon. *S. neomexicana* was frequent on rocky slopes near Limpia Canyon in the Davis Mountains.

TABLE I. *List of Malvaceous Species, Native and Introduced, Collected in Texas, with Notes on Habitat, Occurrence, and Zones*

Species	Habitat	Occurrence	Zones
Abutilon berlandieri Gray	Woods, open chaparral, weed in fields. Somewhat xerophytic. Native.	Corpus Christi, Brownsville, Laredo, Uvalde.	Semi-tropical, L. Sonoran.
A. incanum (Link) Sweet	Woods, open chaparral, rocky hillsides, mountain sides, weed in fields. Fairly xerophytic. Native.	Corpus Christi, San Antonio, Austin, Brownsville, El Paso.	Semi-tropical, L. Sonoran, U. Sonoran.
A. jacquini Don.	Palm woods. Mesophytic. Native.	Brownsville.	Semi-tropical.
A. malacum S. Wats.	Rocky hillsides and mountain sides. Very xerophytic. Native.	Boquillas, Balmorhea, El Paso.	L. Sonoran, U. Sonoran.
A. pedunculare H. B. K.	Palm woods. Mesophytic. Native.	Brownsville.	Semi-tropical.
A. texense T. & G.	Valleys and plains. Xerophytic. Native.	Ozona, Barstow.	L. Sonoran, U. Sonoran.
A. triquetrum (L.) Presl.	Palm woods, very rare in mesquite and huisache. Mesophytic. Native.	Brownsville.	Semi-tropical.
A. wrightii Gray	Rocky hillsides and mountain sides. Rare in valleys. Xerophytic. Native.	Corpus Christi, San Antonio, Laredo, Marfa, Balmorhea.	Semi-tropical, L. Sonoran, U. Sonoran.
A. parvulum Gray	Rocky hillsides and mountain sides. Xerophytic. Native.	Western Texas.	U. Sonoran.
Althaea rosea Cav.	Introduced as a decorative plant.	Throughout Texas.	Semi-tropical, Austroriparian, L. Sonoran, U. Sonoran.
Bastardia viscosa (L.) H.B.K.	Among mesquite and prickly pear. Mesophytic. Native.	Brownsville.	Semi-tropical.
Callirrhoe digitata Nutt.	Woods and thickets. Mesophytic. Native.	Corpus Christi, San Antonio, Del Rio.	Semi-tropical, L. Sonoran.
C. involucrata (Nutt.) Gray	Prairies and woods. Mesophytic. Native.	Hearne, Galveston, Laredo, Sheffield.	Semi-tropical, L. Sonoran, U. Sonoran, Austroriparian.
C. leariloba (T. & G.) Gray	Sandy soil. Mesophytic. Native.	Hebronville.	L. Sonoran.
Cienfuegosia sulphurea (St. Hil.) Garcke	Moist black soil. Mesophytic. Native.	Corpus Christi, Taft.	Semi-tropical.
Disella hederacea (Dougl.) Greene	Alkaline soil. Xerophytic. Native.	Along Rio Grande from El Paso to Presidio.	L. Sonoran.
D. lepidota (Gray) Greene	Plains and along streams. Xerophytic. Native.	Along the Rio Grande in the Big Bend, Pecos.	L. Sonoran.
D. sagittaefolia (Gray) Greene	Weed along streets. Xerophytic. Native.	Alpine.	U. Sonoran.

TABLE I (*Continued*)

Species	Habitat	Occurrence	Zones
Gayoides crispum (L.) Small	Rocky hillsides and mountain sides, in woods along creeks. Xerophytic. Native.	Laredo, Davis Mts., Big Bend.	L. Sonoran, U. Sonoran, Semi-tropical.
Hibiscus cardiophyllus Gray	Mesquite woods, clay banks, sandy and gravelly hills, rocky cliffs, rarely cultivated. Somewhat xerophytic. Native.	Brownsville to Laredo to Uvalde.	Semi-tropical, L. Sonoran.
H. coccineus Walt.	Cultivated. Introduced.	Southeastern Texas.	Semi-tropical.
H. coulteri Gray	Rocky hillsides and mountain sides. Xerophytic. Native.	Del Rio throughout Big Bend to El Paso.	L. Sonoran, U. Sonoran.
H. denudatus var. *involucellatus* Gray	Rocky hillsides and mountain sides. Xerophytic. Native.	From Boquillas and Marathon throughout Big Bend to El Paso.	L. Sonoran, U. Sonoran.
H. esculentus L.	Cultivated.	Throughout Texas.	Semi-tropical, Austroriparian, L. Sonoran, U. Sonoran.
H. lasiocarpus Cav.	Fresh water swamps, cultivated. Swamp plant. Native.	Chambers, Liberty, Jefferson counties.	Semi-tropical, Austroriparian.
H. manihot L.	Cultivated. Introduced.	Austin.	L. Sonoran.
H. militaris Cav.	Fresh water swamps, cultivated. Swamp plant. Native.	Chambers and Jefferson counties, Hearne.	Semi-tropical, Austroriparian.
H. rosa-sinensis L.	Cultivated. Introduced.	Southeastern Texas, San Antonio, Brownsville, El Paso.	Semi-tropical, L. Sonoran.
H. sabdariffa L.	Cultivated. Introduced.	Alvin, Brownsville to Mission.	Semi-tropical.
H. syriacus L.	Cultivated. Introduced.	Throughout Texas.	Semi-tropical, Austroriparian, L. Sonoran, U. Sonoran.
H. trionum L.	Cultivated. Introduced.	Throughout Texas.	Semi-tropical, Austroriparian, L. Sonoran, U. Sonoran.
Kosteletzkya althaeifolia (Chapm.) Gray	Salt water marshes and fresh water swamps near the coast. Swamp plant. Native.	Jefferson, Chambers, and Galveston counties.	Semi-tropical.
Malachra capitata L.	Borders of woods, weed in fields. Mesophytic. Native.	Brownsville.	Semi-tropical.
Malva parviflora L.	Common weed. Introduced.	Brownsville to El Paso.	Semi-tropical, L. Sonoran, U. Sonoran.

TABLE I (*Continued*)

Species	Habitat	Occurrence	Zones
M. sylvestris L.	Cultivated. Introduced.	Throughout Texas.	Semi-tropical, Austroriparian, L. Sonoran, U. Sonoran.
Malvastrum americanum (L.) Torr.	Woods in valleys, common weed. Native. Mesophytic.	Southeastern Texas, Corpus Christi, Brownsville, San Antonio to Del Rio.	Semi-tropical, L. Sonoran.
M. coccineum (Pursh) Gray	Plains. Xerophytic. Native.	Alpine, Great Plains generally.	U. Sonoran.
M. elatum (Baker) Nels.	Sandy and rocky soil. Xerophytic. Native.	Alpine, Davis Mts. to El Paso.	U. Sonoran.
M. spicatum (L.) Gray	Open woods and chaparral, weed in fields. Mesophytic. Native.	Brownsville to Mission.	Semi-tropical.
M. wrightii Gray	Mesquite woods, weed along fences and roads. Mesophytic. Native.	Corpus Christi.	Semi-tropical.
Malvaviscus arboreus Cav.	Cultivated. Introduced.	Brownsville.	Semi-tropical.
M. drummondii T. & G.	Woods and thickets, cultivated. Mesophytic. Native.	Southeastern Texas, Hearne, San Antonio, Corpus Christi, Brownsville, Laredo.	Semi-tropical, Austroriparian, L. Sonoran.
Modiola caroliniana (L.) G. Don.	Rather moist, low places, weed. Mesophytic. Native.	Southeastern Texas, Hearne, San Antonio, Brownsville.	Semi-tropical, Austroriparian, L. Sonoran.
Pavonia lasiopetala Scheele	Dry, rocky banks of ravines. Mesophytic. Native.	New Braunfels.	L. Sonoran.
Sida angustifolia Lam.	Open woods, weed in fields. Mesophytic. Native.	Brownsville to Laredo and Cotulla.	Semi-tropical, L. Sonoran.
S. ciliaris L.	Open mesquite woods, weed in fields and lawns. Mesophytic. Native.	Riviera, Corpus Christi, Gregory.	Semi-tropical.
S. cuneifolia Gray	Sandy soil. Xerophytic. Native.	Brownsville to Laredo.	Semi-tropical, L. Sonoran.
S. diffusa H.B.K.	Dry mesquite woods, plains, sandy and rocky hillsides, mountains and valleys. Xerophytic. Native.	San Antonio, Hearne, Brownsville to El Paso.	Semi-tropical, Austroriparian, L. Sonoran, U. Sonoran.
S. filiformis Moric.	Usually associated with *S. diffusa*. Xerophytic. Native.	San Antonio and Brownsville to El Paso.	Semi-tropical, L. Sonoran, U. Sonoran.
Sida hastata St. Hil.	Woods, plains, rocky and sandy slopes, weed along streets. Somewhat xerophytic. Native.	San Antonio, Brownsville, El Paso, Davis Mts., Pecos.	Semi-tropical, L. Sonoran, U. Sonoran.
S. longipes Gray	Rocky hillsides and mountain sides. Xerophytic. Native.	Sanderson to Marathon.	L. Sonoran, U. Sonoran.

TABLE 1 (*Continued*)

Species	Habitat	Occurrence	Zones
S. neomexicana Gray	Mountain slopes. Xerophytic. Native.	Limpia Canyon in Davis Mountains.	U. Sonoran.
S. rhombifolia L.	Common weed in waste places. Mesophytic. Native.	Southeastern Texas.	Semi-tropical.
S. spinosa L.	Rare in woods, common weed in fields. Mesophytic. Native.	Southeastern Texas, Gregory, Brownsville	Semi-tropical.
S. tragiaefolia Gray	Rocky valleys and mountain sides. Xerophytic. Native.	Alpine. Boquillas.	L. Sonoran, U. Sonoran.
Sphaeralcea cuspidata Gray	Valleys, sandy or clay soil. Weed in fields. Xerophytic. Native.	Eagle Pass to El Paso, Pecos.	L. Sonoran, U. Sonoran.
S. hastulata Gray	Sandy and gravelly valleys and plains. Xerophytic. Native.	Roma to Del Rio.	L. Sonoran.
S. incana Torr.	Rocky mountain slopes, common weed in waste places. Xerophytic. Native.	Davis mountains, Alpine, El Paso.	L. Sonoran, U. Sonoran.
S. lobata Wooton	Sandy loam valleys, rocky slopes, weed in waste places. Xerophytic. Native.	One plant in Brownsville. Pecos, Ruidosa, Alpine, El Paso.	Semi-tropical, L. Sonoran, U. Sonoran.
S. pedatifida Gray	Sandy and gravelly slopes and plains. Xerophytic. Native.	Laredo, Cotulla, Eagle Pass.	L. Sonoran, U. Sonoran.
S. pumila Woot. & Standl.	Sandy mountain slopes. Xerophytic. Native.	El Paso.	U. Sonoran.
S. subhastata Coult.	Valleys and plains, base of mountain slopes. Xerophytic. Native.	Pecos, Sierra Blanca, Shafter.	L. Sonoran.
S. tenuipes Woot. & Standl.	Rocky mountain sides. Xerophytic. Native.	Sierra Blanca, El Paso.	U. Sonoran, L. Sonoran.
Wissadula holosericea (Scheele) Garcke	Rocky hills and mountain slopes. Xerophytic. Native.	Davis Mountains, Balmorhea, Alpine, San Antonio.	L. Sonoran. U. Sonoran.
W. lozani (Rose) Fries	Open woods, weed in fields and along roads. Somewhat xerophytic. Native.	Corpus Christi and Brownsville to Laredo.	Semi-tropical, L. Sonoran.
W. periplocifolia (L.) Griseb.	Open woods, borders of woods, weed in fields and along roads. Mesophytic. Native.	Brownsville.	Semi-tropical.

SUMMARY

1. In this paper several counties in the extreme southeastern part of Texas and the southwestern part of the state from Corpus Christi and San Antonio west to New Mexico were studied.

2. A total of 66 species of Malvaceae were found; 55 of these are native, 9 introduced and found only in cultivation, 1 introduced and found both in cultivation and as a weed, and 1 as an introduced weed. These species are listed in Table 1.

3. In the Semi-tropical Gulf Strip 32 native species were found, in a very small area of the Austroriparian 4, in the large area of the Lower Sonoran 35, and in the Upper Sonoran 22.

4. Classifying these species according to habitat: in open woods in the southeastern part of the state 3 species were found, in southeastern swamps 3, in southeastern prairies 2, in plains 16, in southwestern woods along streams and lakes 21, in southwestern chaparral 19, in palm woods in vicinity of Brownsville only 9, on rocky slopes in west and southwest 22, in alkaline soil in west 2.

5. Semi-tropical species of limited distribution are: *Bastardia viscosa*, *Malachra capitata*, *Abutilon pedunculare*, *A. jacquini*, *A. triquetrum*, *Wissadula periplocifolia*, and *Cienfuegosia sulphurea*.

6. Western species of decidedly xerophytic type are: *Disella* spp., *Sphaeralcea* spp., *Sida* spp., *Abutilon malacum*, *Hibiscus denudatus* var. *involucellatus*, *H. coulteri*, *Malvastrum coccineum*, and *M. elatum*.

7. Eastern mesophytic species are *Hibiscus lasiocarpus*, *H. militaris*, and *Kosteletzkya althaeifolia*.

8. Species of very wide distribution are: *Callirrhoe involucrata*, *Malvastrum americanum*, *Malva parviflora*, *Sida diffusa*, *S. spinosa*, *S. hastata*, *Abutilon incanum*, and *Malvaviscus drummondii*.

UNIVERSITY OF COLORADO,
BOULDER, COLORADO

BIBLIOGRAPHY

1. **Bailey, V.** Biological survey of Texas. Fauna 25, U. S. Biol. Surv. 1905.
2. **Bray, W. L.** The ecological relations of the vegetation of western Texas. Bot. Gaz. **32**: 99. 1901.
3. ——. The vegetation of the Sotol country in Texas. Univ. Texas Bull. **60**. 1905.
4. ——. Distribution and adaptation of the vegetation of Texas. Univ. Texas Bull. **82**. 1906.
5. **Cook, O. F.** Change of vegetation on the South Texas prairies. Bur. Plant Ind. Circ. **14**. 1908.
6. **Coulter, J. M.** Botany of western Texas. Contrib. U. S. Nat. Herb. **2**. 1891–1894.
7. **Havard, V.** Report on the flora of southern and western Texas. Proc. U. S. Nat. Mus. **8**: 449–533. 1885.
8. **Heller, A. A.** Contrib. Herb. Franklin and Marshall Coll. **1**: 67. 1895.
9. **Hill, R. T.** Physical geography of the Texas region. U. S. Geol. Surv. Topograph. Atlas U. S. 1900.
10. **Lewis, I. M.** The trees of Texas. Univ. Texas Bull. **22**: 1915.

11. **Lewton, F. L.** *Cienfuegosia drummondii,* a rare Texas plant. Bull. Torrey Bot. Club. **37**: 473–475. 1910.

12. **Merriam, C. H.** Life zones and crop zones of the United States. U. S. Biol. Surv. Bull. **10**: 1898.

13. **Palmer, E. J.** The canyon flora of the Edwards plateau of Texas. Jour. Arnold Arboretum **1**: 233–239. 1920.

14. **Small, J. K.** Flora of Southeastern United States. 2nd ed. New York, 1913.

15. **Stearnes, F.** Flora de la region norte de Chihuahua. Estac. Agric. Exper. Ciudad Juarez Bull. 38. 1912.

16. **Udden, J. C., Baker, C. L., and Bose, E.** Review of the geology of Texas. Univ. Texas Bull. **44**. 1916.

17. **Wooton, E. O., and Standley, P.** Flora of New Mexico. Contrib. U. S. Nat. Herb. **19**. 1915.

NOTE ON THE HISTOLOGY OF GRAIN ROOTS

Grace A. Dunn

(Received for publication December 6, 1920)

In the winter of 1920, Dr. Sophia Eckerson and the writer were engaged in a microchemical study of the physiology of the absorption of different salts by plants grown in water cultures, especially *Zea mays* (white dent) and *Triticum vulgare.* The experimentation was carried out in Dr. Rodney H. True's laboratories in the U. S. Bureau of Plant Industry, at Washington. In this work it was observed that roots of Indian corn and wheat grown in solution cultures showed an interesting structural feature that has apparently not yet received attention in the literature. Openings appeared in the root cortex, the time and place of their appearance varying with the plant and with the solution used. No serious study of the causes involved in the formation of these openings was attempted, but some histological observations were made as to the general manner of their occurrence. Hand sections of the roots were used. Since it was necessary to discontinue these studies for the time being, this note has been prepared in order to present the information which has thus far been gained.

The seeds were germinated between sheets of filter paper moistened with distilled water, and the seedlings were placed in the solutions when the roots were 1–3 cm. long. Some cultures were maintained in darkness at a temperature of about 18° C., while others were under laboratory conditions at a temperature between 17° and 22° C. Tests showed that the solution concentration varied considerably during an experiment; the solution was not changed during the culture period, 7–14 days. Single-salt solutions of $Ca(NO_3)_2$, $Mg(NO_3)_2$, KNO_3, $CaSO_4$, $MgSO_4$, and K_2SO_4 were employed, each with 0.00024 normal concentration. Also, both plant forms were grown in 3-salt solutions containing KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$, with a trace of $FePO_4$. Openings were found in plants of both species grown in each of these solutions, these openings appearing in the corn roots after 3 or 4 days, much sooner than in the case of wheat. They appeared in wheat roots, in all the single-salt solutions, after 8 days, developing more rapidly in solutions with the calcium salts than in those with either the magnesium or the potassium salts.

As to the openings themselves, when they are first observed they are of about the size of the adjoining cortical cells, as shown in figure 1, and appear like large intercellular spaces formed by a separation of adjacent cells in the cortical tissue. In later stages the openings appear larger, and there is evidence (at least in the case of corn) that some cells have broken down, as if the openings had been enlarged through the disintegration of adjoining cells. In corn roots with well developed openings, fragments of cell walls are often seen projecting into the openings, as shown in figure 3. In a wheat root that has been growing fairly rapidly for a period of eight days, the openings are 1 or 2 cortical cells in diameter and are nearly spherical. At a later stage about nine openings are seen in a cross section of the root, separated by septa composed of one or two layers of unmodified cortical cells.

It was thought that this occurrence might be due to lack of proper physiological balance in the nutrient medium. The single-salt solutions each contained only two of the six essential elements, and there was no evidence that the 3-salt solutions employed were well balanced; moreover they had not been renewed as frequently as is customary in water-culture work. This suggestion was tested by employing Shive's solutions R1C2 and R2C5 (optimal series), which are to be regarded as at least fairly well balanced for wheat. The solutions were renewed on the fourth day and the cultures were continued for 10 days, beginning March 1. Openings appeared just as before, being visible on the fifth day and well developed on the seventh.

Beginning about the middle of June a few experiments were carried out for the sake of further observations on the openings in question. Cultures were grown in Shive's solution R5C2 (sub-optimal series), in a good garden soil and in sand. Marquis wheat, from the lot of seed used in the cooperative project of the Division of Biology and Agriculture, of the National Research Council[1] was used, and the water-culture seedlings were supported by perforated cork stoppers in pint "Mason" jars.

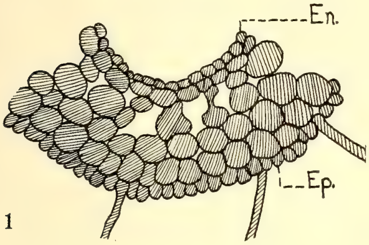
The solution cultures were in the greenhouse at temperatures varying between 25°–45° C. Some of the solutions were renewed daily for the first three days, but this treatment seemed to exert no influence on the formation of the openings, which appeared at about the same time and in the same way in all cultures.

Rather large intercellular spaces were observed on the fifth day. Two days later a cross section of the region 4–6 cm. below the seed appeared as is shown in figure 1. On the ninth day the openings were larger and presented the appearance shown in figure 2. The roots were then 18 cm. long and openings were present 2 to 14 cm. below the seed, those toward the tip of the root being smaller. The smaller openings certainly contained gas. All the plants of these solution cultures appeared to be healthy and vigorous throughout the experiment; on the thirteenth day, when the cultures were discontinued, the shoots were about 19 cm. high.

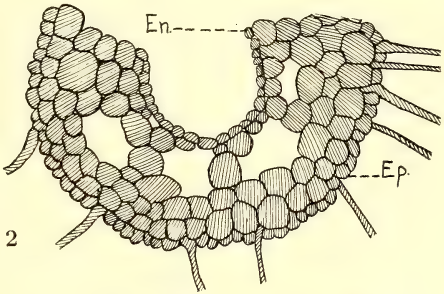
The sand and soil cultures mentioned above were continued for 13 days in the greenhouse, at temperatures between 25° and 45° C., the media being kept as moist as in ordinary greenhouse cultures. At the end of this time the shoots were about 15 cm. high, while the roots were about 15 cm. long. There was no indication in any of these roots of openings in the cortex. The plants grew somewhat less rapidly in the soil and sand cultures than in the solution cultures, but the roots in the sand and soil cultures were as long at the end of the experiment as were those in the solutions when pronounced openings were noticeable.

---

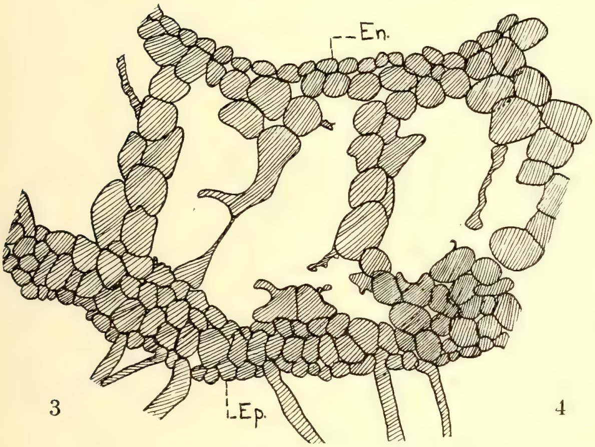
[1] Livingston, B. E. A plan for cooperative research on the salt requirements of representative agricultural plants. Baltimore, 1919.



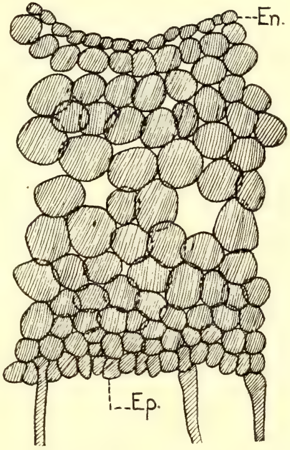
1



2



3



4

FIG. 1. Transverse section of wheat root, 4 cm. below seed. Plant grown 7 days in 3-salt solution.

FIG. 2. Transverse section of wheat root, 4 cm. below seed. Plant grown 9 days in 3-salt solution.

FIG. 3. Transverse section 3½ cm. below seed of corn root 26 cm. in length. Plant grown 8 days in garden soil.

FIG. 4. Transverse section 3 cm. below seed of corn root 18 cm. in length. Plant grown 8 days in garden soil.

En. endodermis; *Ep.* epidermis.

Summarizing all the results so far available for wheat, the openings here considered were regularly obtained in roots grown in solution, whether the latter were fairly well balanced or decidedly unbalanced and whether the plants were grown under winter or summer greenhouse conditions.

The openings do not appear to have been related to the presence or absence of any chemical element or salt in the solution. But wheat roots failed to exhibit these cortical openings when grown in sand or soil for 13 days at summer greenhouse temperature. Whether wheat may be expected to show these openings always when grown in liquid media, and whether it may sometimes show them when grown in soil or sand, remain open questions.

Turning to the observations on Indian corn, during the months of January, February, and March the white dent variety was grown in many different solutions (with from one to five salts), and cortical openings were regularly developed in roots that had reached a length of 10 or 12 cm. Plants of the same variety grown in moist, rich garden soil within the same period uniformly failed to show openings in roots 10 to 18 cm. in length.

In June, Shive's solution R4C5 (sub-optimal series) produced openings like those shown in figure 3. The seeds had been germinated in sphagnum (15°–35° C.), and the seedlings had been in the solution (20°–35° C.) for only 70 hours. The openings were present to within about 3 cm. of the tip of the root. At about the same time similar results were obtained with distilled water and with single salt solutions (0.00048 normal) of $Ca(NO_3)_2$ and $Mg(NO_3)_2$. Apparently the liquid media produced the same growth response in these roots in summer as in winter.

With soil and sand cultures, however, the results obtained in June were somewhat different from those recorded for the winter and early spring. Corn plants that had grown 12 days in moist, rich garden soil (greenhouse, 20°–40° C.) showed many cortical openings, even to within 3 cm. of the tip of the root (fig. 3). The openings in the older portion of the root appeared very large in cross section and were about 8 cells in length. Perfectly parallel results were obtained with moist sand cultures at the same time.

White dent corn seedlings that had been grown in sphagnum (in the greenhouse) until the roots were 10 cm. long showed pronounced openings in the older portions of the root (to 4 cm. below the seed) when the test was made in June, but showed no openings in the case of similar tests made in the winter months.

It appears that cortical openings were regularly formed in white dent roots more than 10 or 12 cm. in length, grown in liquid media, whether in winter or in summer, and they also appeared in roots 12, 14, or 16 cm. in length, grown in soil, sand, or sphagnum during a period of high temperatures in the summer. The rate of growth of the root rather than the age of the seedling seems to be the factor determining the time of appearance of these openings. It is at once suggested that temperature and oxygen supply may be among the environmental conditions influencing the phenomenon in question. It seems safe to suppose that roots grown in sand, soil, or sphagnum generally have better opportunity for absorbing oxygen than those grown in liquid media, and this consideration may possibly furnish an explanation of the difference observed in winter, when the temperature was relatively low and when growth, respiration, etc., were not as rapid as in summer. It appears as if the higher temperatures of summer, with higher respiratory activity, etc., may have rendered the better aerated, solid-medium cultures more like those in liquid media.

Several other experiments, not here described, seemed to support the idea that temperatures that gave slow growth in soil cultures retarded or prevented the development of the cortical openings, while temperatures that gave rapid growth resulted in numerous well developed openings. This suggestion seems to be along somewhat the same line as that expressed by Laetitia M. Snow[2] regarding the formation of gas cavities in the stems of Scirpus.

It is to be noted, however, that the development of cortical openings in corn roots frequently occurs after the root tissue has ceased to elongate. These openings may form, apparently, either in the elongating region or in the older part of the root where elongation has stopped.

One other experiment is worthy of mention here. On June 12 two soil cultures of white dent corn were prepared, one being placed in the greenhouse (higher temperatures) and the other in the open (lower temperatures). At the end of 8 days the greenhouse culture had roots about 26 cm. long, with large cortical openings (as in fig. 3) throughout the older portion, to within about 5 cm. from the tip. The openings in the upper part of the root were larger than those nearer the tip. At the same time the out-door culture had roots only about 18 cm. long, with all the pronounced openings (as in fig. 3) confined to the 3 cm. just below the seed. In the remainder of the root occurred only very small openings, merely intercellular spaces such as those shown in figure 4.

General Conclusion. It appears that the cortical openings considered in this note generally appear in solution cultures of both Marquis wheat and white dent corn, under greenhouse conditions of both winter and summer. They were not observed in the sand or in the soil cultures of wheat in either winter or summer, but none of these cultures were continued for more than thirteen days. The time of appearance of the cortical openings in sand or soil cultures of the white dent corn seems to vary with the rate of growth of the roots.

WASHINGTON, D. C.

[2] Snow, Laetitia M. Diaphragms of water plants. II. Effect of certain factors upon development of air chambers and diaphragms. Bot. Gaz. 69: 297–317. 1920.

NORTH AMERICAN PIPERS OF THE SECTION OTTONIA[1]

William Trelease

(Received for publication December 9, 1920)

The woody Piperaceae, with 2–5 unfimbriate stigmas, comprise the better part of 1,000 species, the extremes of which differ greatly while the segregation of those that are closely related is very difficult. The structure and numerical plan of the minute flowers run over so large a range of differences that it seems quite unreasonable to include them all in a single genus; but however the lines be drawn, the segregates remain too heterogeneous and laxly characterized to give satisfaction. Even as subgenera or sections of the single genus Piper, this is true of them.

When Casimir de Candolle monographed the family, half a century ago, he admitted several such subdivisions of Piper (1), and with various emendations these are kept up in most of his publications during the two generations through which he has been accorded by common consent the title of master in this field. Shortly before his death, however, in a manuscript (2) on the Central American representatives of the genus, his expressed views on this point underwent modification to the extent of assigning to a distinct section, Ottonia, the species with pedicellate flowers which he had placed formerly partly in the section Enckea and partly in the section Steffensia. This section corresponds to Sprengel's genus Ottonia, of 1820; the type of which is the South American *Piper Jaborandi.*

Though M. de Candolle monographed the West Indian Piperaceae (3) eighteen years ago and subsequently added descriptions of occasional novelties, and made a reexamination of the Isthmian and Central American species of Piper when preparing the manuscript of which parts have been published within the past year (4), no effort has been made to consider the Mexican species collectively since Hemsley included them in his tabulation of the Central American flora nearly forty years ago. Indeed, in the whole large genus, only two Mexican species (5) have been described during the past generation.

Among a considerable number of undetermined Pipers at the New York Botanical Garden and in the United States National Herbarium, which Dr. Britton and Mr. Maxon have permitted me to study preliminary to a general revision of the North American representatives of the family, several interesting Mexican novelties in the pedicellate section Ottonia occur. The analysis of these which follows may serve to illustrate the statement made earlier, that however simply a section may be conceived, in Piper, it is certain to prove heterogeneous in what are regarded ordinarily as significant floral characters.

---

[1] Read before the Taxonomic Section of the Botanical Society of America, at Chicago, December 29, 1920.

As here construed, excluding the Javan *Piper Zippelia*, the Ottonias are exclusively American. Two thirds of their number occur in South America; and one appears to be limited to the continental island Trinidad. Of the others, eight are Mexican: one in the lowlands of Yucatan, two in the Eastern Sierra Madre and Cordillera, and five in the Western Sierra Madre. One species is Honduranian, and one Nicaraguan.

Except for the Trinidad species, which, like those of continental South America, has pinnately veined leaves, all of these have palmately nerved foliage. In contrast with those of the eastern slope, which have elongated, rather lance-ovate leaves, those of western Mexico bear round-ovate, often shallowly cordate-truncate leaves.

In the diagnostic stalking of their flowers they present a gradation from the close-set, nearly sessile flowers of *P. brachypus* to those with a pedicel distinctly longer than the flower.

Characteristically hypogynous, the stamens in two species are adnate to the ovary for a considerable distance; in this respect paralleling the sessile-flowered Pipers, some of which are quite hypogynous while others have epigynous stamens. In one of the Ottonias, *P. abalienatum*, the stamens form two separated alternating whorls around the ovary.

Though the stigmas are essentially sessile on the ovary in species like *P. Muelleri* and the epigynous *P. albicaule*, the fruiting ovary is attenuate into something of a beak in the former; and *P. abalienatum*, even when in flower, possesses a columnar style essentially as long as the ovary. Perhaps the most interesting species in this respect is *P. brachypus*, in which a very short, thick style matures into a stylopodial disk which caps the fruit and is comparable with that of the sessile-flowered *P. smilacifolium*—one form of which was segregated formerly under the name *P. discophorum*.

CONSPECTUS OF THE NORTH AMERICAN OTTONIAS

Leaves pinnately nerved.	*Piper ovatum.*
Leaves palmately nerved.	
Leaves distinctly longer than broad.	
Leaves somewhat pubescent beneath.	*P. Muelleri.*
Leaves and petioles glabrous or barely puberulous.	
Leaves broadly ovate.	*P. yucatanense.*
Leaves lance-ovate: spikes rather short.	
Leaves acute and minutely subauriculate at base.	*P. Neesianum.*
Leaves rounded at base.	
Spikes scarcely 20 mm. long.	*P. Thiemeanum.*
Spikes 25–30 mm. long.	*P. Tatei.*
Leaves about as broad as long.	
Pedicels very short.	*P. brachypus.*
Pedicels very evident.	

Stamens hypogynous.	
Leaves glabrate.	
Spike not longer than leaf.	*P. Diguetianum.*
Spike distinctly longer.	*P. Mas.*
Leaves puberulent; spike elongated.	*P. Rosei.*
Stamens adnate to ovary: leaves pubescent.	
Style very prominent.	*P. abalienatum.*
Style nearly suppressed.	*P. albicaule.*

Piper ovatum Vahl, Eclog. 3. Pl. 1. 1796.—C.DC., Prod. **16**^{1}: 253, and Urban, Symbolae Antillanae **3**: 174.

Glabrous; leaves ovate-elliptic, acuminate, narrowed and nearly equally cordulate at base, moderately small (5–6.5 × 12–14 cm.), pinnately nerved throughout, the nerves 10 or 12 × 2; petiole short (10 mm.); spikes moderately short (50 mm.); peduncle equaling the petiole; bracts concave; flowers short-pediceled, perfect, hypogynous, 4-androus; ovary with a short style; stigmas 4; berries ovoid-attenuate.

Trinidad, West Indies (*Ryan*, the type; *Purdie; Fendler 669;* not known to be represented in North American herbaria).

Piper Muelleri C.DC., Prod. **16**^{1}: 243. 1869.

More or less soft-pubescent; leaves elliptic-ovate, acuminate, rounded at base, small (scarcely 5 × 12 cm.), palmately 5- or 7-nerved, bullate in age; petiole short (5–10 mm.); spikes moderately short (50 mm.); peduncle exceeding the petiole (15 mm.); bracts concave; flowers pedicellate with the pedicels unequally long up to 1.5 mm., perfect, 6-androus, with hypogynous anthers rather shorter than the filaments; stigmas 3, sessile; berries round-ovoid, apiculate.—Plate V, figure 1.

Eastern Sierra Madre, Mexico, about Orizaba (*Mueller 180*, the type; *Botteri 1156*).

Piper yucatanense C.DC., Linnaea **37**: 334. "1871–3."

Closely resembling *P. Muelleri* but glabrous throughout, the subsessile leaves ovate and measuring 6.5 × 12 cm.

Yucatan, Mexico (*Linden 184:* not known to be represented in American herbaria.)

Piper Neesianum C.DC., Prod. **16**^{1}: 256. 1869.

Glabrous except that the leaves are minutely puberulous beneath; leaves lance-elliptic, long-acuminate, acute and minutely unguiculate-auriculate at base, small (scarcely 4 × 10 cm.), palmately 5-nerved; petiole moderate (10 mm.); spikes very short (scarcely 20 mm.); peduncle half as long as the spike; bracts evanescent; flowers pedicellate with the pedicels scarcely over 1 mm. long, 3- to 6-androus, hypogynous; ovary ovoid or oblong-ovoid; stigmas 3, sessile.—Plate VII, figure 1.

Eastern Sierra Madre, Mexico (without indicated locality, *Karwinski 823*, the type); Papantla (*Liebmann 18*); Orizaba (*Botteri 192*). Also reported from Nicaragua (*Tate 367*).

Piper Thiemeanum n. sp.

Glabrous; leaves ovate-lanceolate, long-acuminate, rounded at base, small (2–4 × 8.5–13 cm.), palmately 5-nerved; petiole very short (3 mm.); spikes very short (scarcely 20 mm.); peduncle nearly half as long as the spike; bracts spatulate; flowers pedicellate with the pedicels unequally long up to 2 mm., 5-androus with large subsessile hypogynous anthers; ovary conical-ovoid, somewhat constricted at apex; stigmas 3.—Plate V, figure 2.

Northern Honduras, about San Pedro Sula (*Thieme 5455*, the type).

Piper Tatei n. sp.

Piper Neesianum Auct., as to Nicaragua.

General characters of *P. Thiemeanum* but the leaves minutely puberulous beneath and the spikes one-half longer (25–30 mm.).

Nicaragua, presumably from Chontales (*Tate 367*, 1867–8, the type at Kew).

Piper brachypus n. sp.

Glabrous, or at most puberulent; leaves somewhat obliquely round-ovate, blunt-acuminate, rounded or subtruncate or shallowly cordate at base, in either case with a short deltoid contraction into the petiole, small (5–6 × 7–8 or even 8 × 9 cm.), palmately about 9-nerved; petiole rather short (5–15 mm.); spikes elongated (80–90 mm.); peduncle short (10–15 mm.); bracts concave, pubescent; flowers short-pediceled with the pedicels scarcely 0.5 mm. long, hypogynous, 5-androus, the filaments very short; ovary somewhat contracted into a short stylopodium; stigmas 3 or 4, subsessile; berries round-ovoid, crowned by the rather evident broad stylopodium.—Plate VI.

Western coast region of Mexico, about Manzanillo (*Palmer 1332*, the type).

Piper Rosei C.DC., in herb., n. sp.

Puberulent; leaves somewhat obliquely round-ovate, acuminate, rounded or shallowly cordate at base with a short deltoid contraction into the petiole, small (6–8 × 8–10 cm.), palmately about 7-nerved; petiole rather short (10–15 mm.); spikes elongated (60–90 mm.); peduncle short (5–10 mm.); bracts concave, puberulous; flowers short-pediceled with the pedicels scarcely 1 mm. long, hypogynous, 3- or 4-androus, the filaments shorter than the rather large anthers; ovary ovoid; stigmas 3, sessile.

Western Sierra Madre of Mexico (Colomas, *Rose 3234*, the type, and *1657*).

Piper Diguetianum n. sp.

Transiently puberulous, or glabrous, with the general characters of *P. Rosei* but the spikes shorter (40 mm.), the pedicels reaching a length of 1.5 mm., and the flowers 5-androus.

Western Sierra Madre of Mexico (Jalisco, without other data, *Diguet*, the type).

Piper Mas n. sp.

Rather transiently puberulent; leaves round-ovate, acuminate, truncate-cordate with short deltoid contraction into the petiole, small (5 × 6 cm.), palmately 7- or obscurely 9-nerved; petiole short (10 mm.); spikes elongated (70–90 mm.); peduncle short (5–15 mm.); bracts concave, glabrate; flowers short-pediceled, with the pedicels scarcely 1 mm. long, hypogynous, 5-androus, the filaments shorter than the rather large anthers; ovary minute, ovoid; stigmas 3, sessile.—Plate VII, figure 2.

Western Sierra Madre of Mexico (El Muleto, *Langlassé 215*, the type).

Piper abalienatum n. sp.

Dingy-villous or pubescent throughout, the puberulous older branches pale; leaves round-ovate or subquadrate, short-acuminate, truncately rounded or shallowly truncate-cordate at base, comparatively large (7.5 × 7.5–16 × 18 cm.), palmately 9- or 11-nerved; petiole rather short (15 mm.); spikes elongated (130 mm.); peduncle rather short (15 mm.); bracts spatulate; flowers rather long-pediceled, the pedicels up to 2 mm. long, 5- or 6-androus, the short filaments adnate nearly to the middle of the flask-shaped ovary; stigmas 3, on a constricted style nearly as long as the ovary; berries subglobose, surmounted by the stout style.—Plate VIII, figure 1.

Western Sierra Madre of Mexico (Colima, *Palmer 100*, the type).

Piper albicaule n. sp.

More or less persistently gray-tomentose or pubescent throughout, branches for a time silvery from the detaching epidermis; leaves subquadrate-orbicular, rather abruptly long-acuminate, truncate at base, rather small (5.5 × 7–8.5 × 9 cm.), palmately about 9-nerved; petiole short (10 mm.); spikes elongated (100 mm.); flowers long-pedicellate, the pedicels up to 2 mm. long, 5- or 6-androus, the very short filaments adnate nearly to the middle of the ovoid ovary; stigmas 3, on a conical style half as long as the flowering ovary but becoming obliterated as the subglobose berry enlarges.—Plate VIII, figure 2.

Western Sierra Madre of Mexico (Santa Rosa near Aguila, *Langlassé 248*, the type).

The University of Illinois,
Urbana

REFERENCES

1. **De Candolle, C.** In DC., Prodromus 16^1: 240. 1869.
2. ——. A manuscript of 64 pages, with an exhaustive supplementary key to 232 species; for the privilege of using which I am indebted to Mr. W. R. Maxon of the United States National Herbarium.
3. ——. In Urban, Symbolae Antillanae **3**: 159. 1902.
4. ——. Smithsonian Miscell. Coll. 2579. 1920. Botanical Gazette **70**: 169. 1920. A key to the recorded Mexican species is included by Mr. Standley in his recently published Trees and Shrubs of Mexico in Contr. U. S. Nat. Herb. 23^1: 145.
5. **Piper jaliscanum** Sereno Watson, Proc. Amer. Acad. **26**: 145. 1891.
Piper Palmeri C. DC. Contr. U. S. Nat. Herb. **1**: 354. 1895.

EXPLANATION OF PLATES

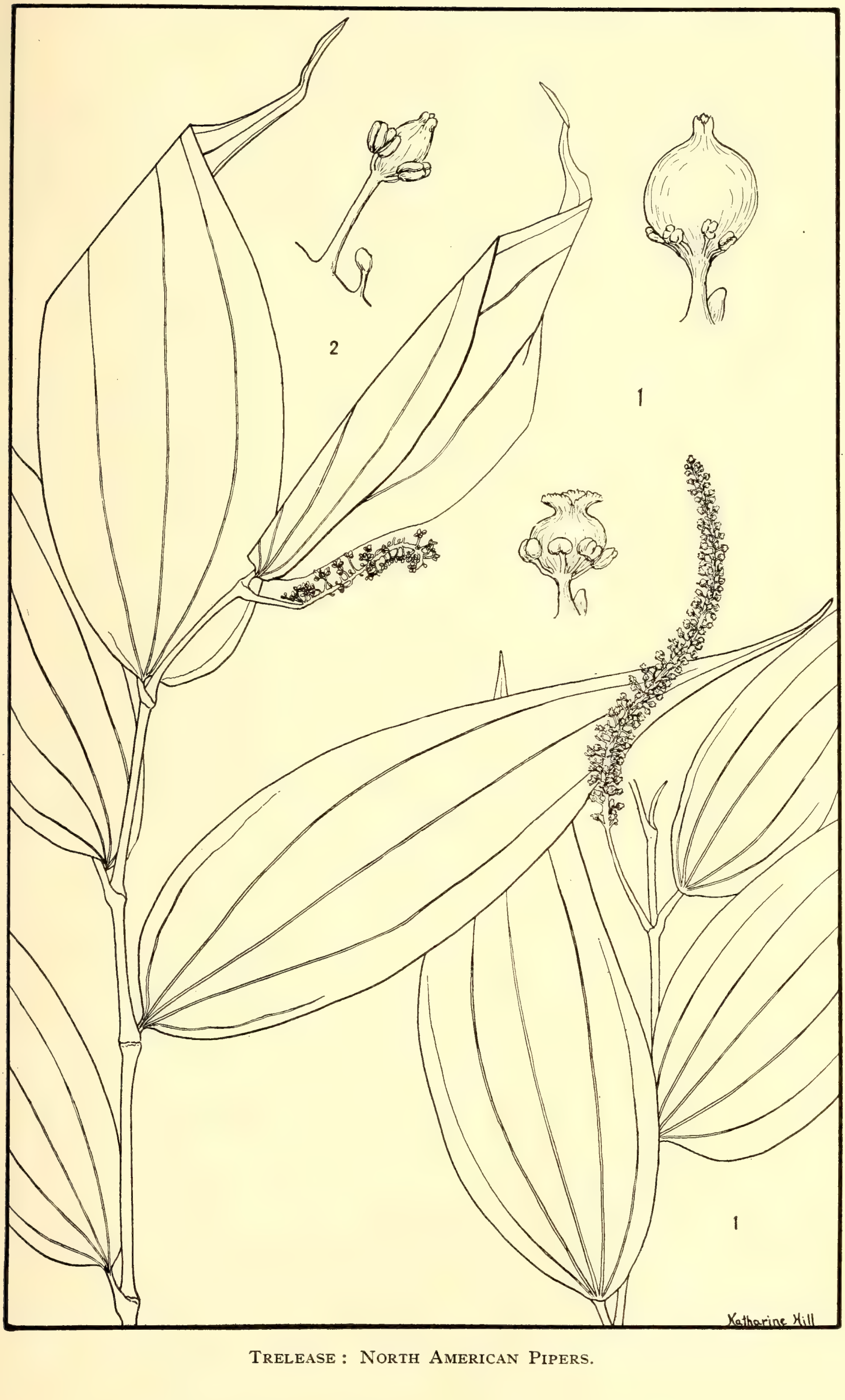
Habit figures are of natural size; flowers or fruit × 10.

Plate V

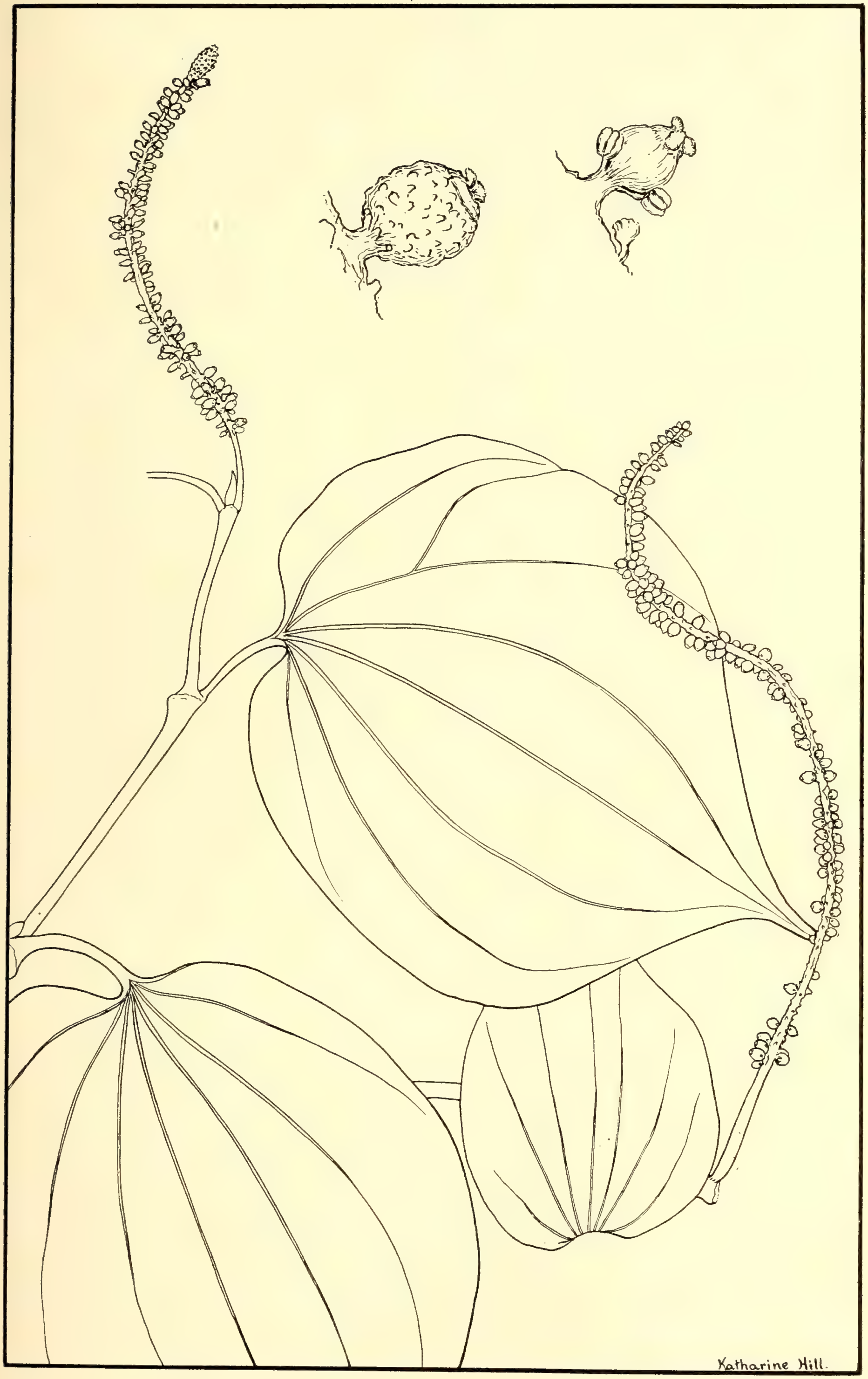
Fig. 1. *Piper Muelleri*, the type collection.
Fig. 2. *Piper Thiemeanum*, the type collection.

Plate VI

Piper brachypus, the type collection.

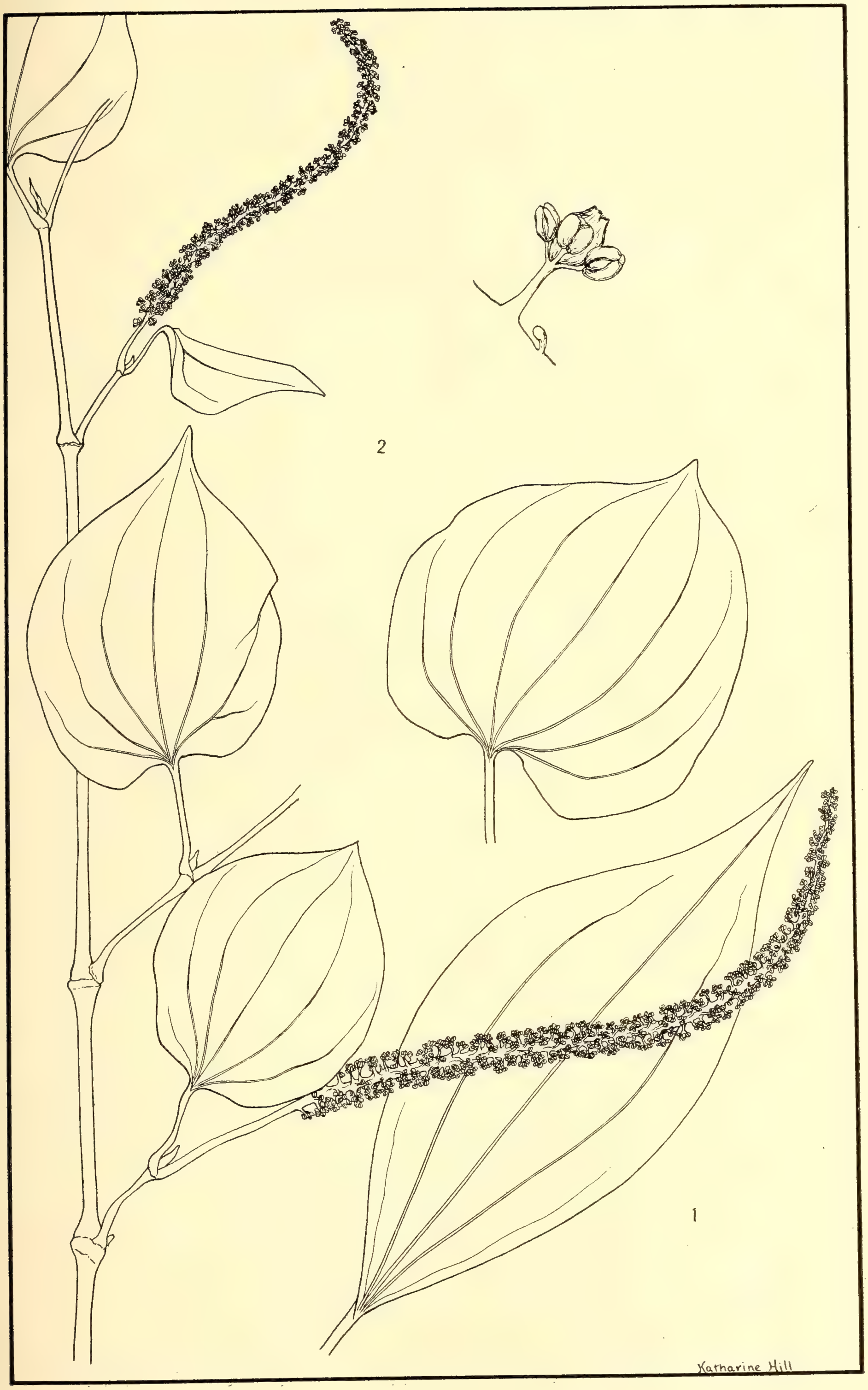

2
1
1
Katharine Hill

TRELEASE: NORTH AMERICAN PIPERS.

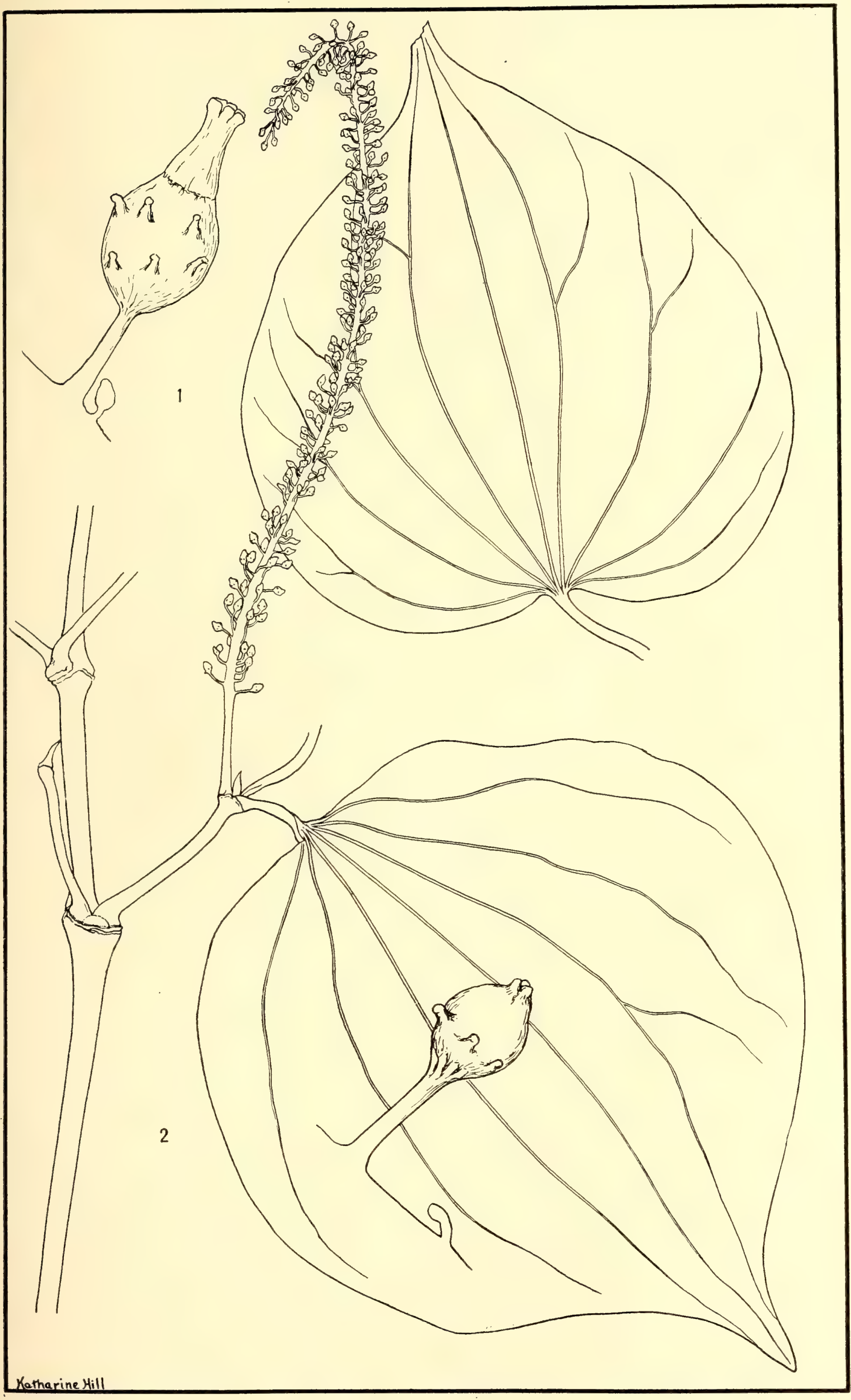


Katharine Hill.

TRELEASE: NORTH AMERICAN PIPERS.


2
1
Katharine Hill

TRELEASE: NORTH AMERICAN PIPERS.


1
2
Katharine Hill

TRELEASE: NORTH AMERICAN PIPERS.

Plate VII

Fig. 1. *Piper Neesianum* (Liebmann 18).
Fig. 2. *Piper Mas*, the type collection.

Plate VIII

Fig. 1. *Piper abalienatum*, the type collection.
Fig. 2. *Piper albicaule*, the type collection.

MONOCARPY AND PSEUDOMONOCARPY IN THE CYCADEOIDS

G. R. Wieland

(Received for publication December 24, 1920)

Seed plants which fruit once only in the normal lifetime, and then die down to make way for the new set, are described as monocarpic. The habit is of much interest to students of ecology and succession.

As ordinarily used, monocarpy is a rather fixed term which requires some extension, or even redefinition. In a stricter sense, the field of rye is monocarpic; though the usage is far less definite in its common application to plants much longer lived than annuals or biennials. There would, however, be a double reason for not calling a "bracken fern" monocarpic; since after the fertile frond bears its *spores*, the root-stock grows on, propagation being only secondarily dependent on either the sexual or the asexual generation. And somewhat similarly, any seed plant which, after the wilting down of a fertile stalk of one or more seasons, renews its growth from the root, is falsely monocarpic. It is an inadvertence on the part of botanists to cite the "century plant" as truly monocarpic, or any plant which does not fixedly persist by reseeding, or solely so persist.

There appear to be some basic physiologic distinctions or phases in monocarpy. The Agave (or *Yucca filamentosa*, which regularly sends up the new buds, with very little germination of seed) does store the materials for the fertile shoot. But in such plants the length of the vegetative phase may be variable, or doubtless even local and individual. Besides, wherever the basal buds grow forward, there is some analogy to the fertile axis of limited growth in trees.

The production of flowers or fruits usually means the growth of peduncular tracheidal or other structures old in the history of the plant, supplying greatly modified or reduced sporophylls; so that fruit maturity may mean, not merely exhaustion of the parent stem, but a heavy scar or injury to the stem not easily survived. Thus as a plant is modified from age to age, and the gap between fruit and vegetative structures widens, proliferation of the fertile axis may become difficult or quite impossible. And such has in fact become the condition in the vast majority of seed plants with renewed growth from the base of flower or cone. Particularly in the conifers the occasional appearance of proliferate cones may be regarded as reminiscent of a time when there were some normally proliferate types in the ancestral series.

Contrariwise, the budding power of stems divested of their foliage or cut down to the base, is often striking in the extreme, especially in some of the Pandanales because little expected. Renewed growth from stem-base or root may then be a characteristic of singular or extreme types. It may even accompany gigantism.

Undoubtedly there is a less known side to monocarpy and the monocarpic tendency; while even in the commoner instances, variations either individual or within the habitat, and details concerning any subsequent root branching, may fail. The classical example is of course the "umbrella palm" of Ceylon, *Corypha umbraculifera.* After a vegetative period of towards forty years the mature height of sixty feet is reached, when in a single short season the stem shoots forty feet higher as a much branched flowering stalk. Meanwhile the foliage fronds wilt down and leave this immense terminal inflorescence with a branch spread of thirty feet, bearing tens of thousands of flowers.[1]

This unparalleled gigantism, however, scarcely exceeds in outright interest a Jamaica plant known as the "pride of the mountain," *Spathelia simplex*, a member of the rue family. For this plant is nearly related to hardwood perennials like Citrus, and the Rutaceæ are not so generally monocarpic. The slender trunk, scarred by the fallen leaves, reaches a height of fifty feet, bearing an apical cluster of large, velvety, pinnate leaves three or four feet long. The pinnules are numerous (45–81), sessile, oblong-lanceolate, crenate. At maturity a large terminal panicle of showy purple flowers rises above the leaves, the plant dying down after the ripening of fruit. There is thus afforded an example of a tall and quite typical dicotyl of much the same habit and habitus as the "umbrella palm."

The primary form of monocarpy finds a notable variation along ecologic lines in the greatest of the grasses, the bamboos. The life cycle in the bamboos varies greatly. Many species bloom annually; and there are also cases of sporadic flowering, with, however, the final production of flowers on all the culms—ripening of seed then terminating the life of the plant (*Arundinaria Simoni*). In the bamboos of the south Brazilian provinces of Santa Catarina and Rio Grande do Sul, along the borders of the imposing *Araucaria brasiliana* forest, a thirteen-year monocarpic period occurs. For *Bambusa tesselata* in cultivation a not very authentic record of a sixty-year cycle is given.

Floral periodicity is well attested in the Indian (Ganges) *Bambusa arundinacea*, reported in flower in the years 1804, 1836, 1868, [1900]. In the Blue mountains of the Island of Jamaica at an altitude of 4,000 to 7,000 feet over a region ten miles long, occurs the "climbing bamboo" (*Chusquea*

---

[1] All the genus Corypha is perhaps more truly monocarpic than are other so-called instances. Root shoots are not sent up. There are two Ceylon species, and other representatives in Bengal and farther eastward. In this connection there should be added the interesting case of the Mauritius hemp, *Furcraea americana*, an agave-like plant. Suckers are not readily produced, but any such flower at the same time as the parent stock. Propagation is mainly by bulbils after flowering.

abietifolia). Here, extending over more than a year in 1885–6, flowering and dying down were general; this occurred again in 1919, when 98 percent of the plants formed the dead entanglements, and the young plants 18 inches high covered the ground in long stretches (as recently described in this Journal).[2] A rough thirty-three-year period is indicated, with only minor or suppressed seasonal factors.

These are communal forms, and whoever has seen one of the isolated dead pure stand thickets of bamboo in an open hilly region, as in parts of southern Mexico (Oaxaca), where there is a long, dry winter period, understands that average length of life and single flowering are complementary, intensified habits. In such plant communities the life of the individual is simple, set, and tense; and as soon as a considerable number of the plants fruit and die down, the light, heat, moisture, and soil conditions of the copse change rapidly. Only the plant behaving in the average way tends to leave its progeny. Even in the tropics there are forms which flower over wide areas on precisely the same day. Then, in the forests of Pegu, certain orchids are seen to blossom as the limbs on which they are seated lose their leaves. Yet there is the remarkable variation in that some trees bloom quite through the year, as the mango, silk cotton, and fig. This has been noted especially in orchids.

Casually, this much may be said of the monocarpic habit. Plants seemingly take full advantage of their environment in reaching their many forms. But they grow as they may, and reproduce as they must. The law is simple, complex though its expression may appear. Reproduction is sharply seasonal in the severe environment, and of more varied phase in the soft climate where growth factors find their favorable mean. In the cool temperate zones, the period of flowering varies for weeks as moisture and heat vary with the unusual season. In the tropics the utmost variations are found. Many of the woody plants are not dependent on their foliage in blossoming in or after the dry season. Such is the habit of various magnolias of the subtropics, and further north, where the burst of flowers is put forth earlier than the young leaves easily grow. These are tender and in our climate whipped by chill winds of early spring. Yet other trees are, as Schimper notes, evergreen in their flowerless youth, losing their foliage as they flower and fruit. Though such (*Schizolobium giganteum* of Java) suggest a certain advance toward monocarpy.

Evidence of Monocarpic Conditions in Cycadeoids

In few extinct plants could we hope to detect evidence for so recondite a feature as flower growth but once in a lifetime, or for any modified form of monocarpy—not even under most favorable circumstances of fossilization. Among all the plants of the past, the petrified cycadeoids alone present the evidence for monocarpic tendencies and pseudo-monocarpy in the numberless fruits, from the youngest stages of growth to the mature seed cones held securely packed between the leaf bases forming the heavy protecting outer "armor" of the short, robust trunks (note Plate IX).

---

[2] Seifriz, William. The length of the life cycle of a climbing bamboo. A striking case of sexual periodicity in *Chusquea abietifolia* Griseb.

Amer. Jour. Bot. 7: 83–94. 1920.

It was first observed in studying the petrified stems from the Black Hills that a tendency to monocarpy was present. In fact, this was considered fairly proven in those instances in which the imbedded young axillary fruits were found throughout the armor. Thus far five of the species of Cycadeoidea are viewed as more or less completely monocarpic:

1. In the trunk fragment from northwest of the Grapevine Valley, Colusa County, California, the axils of the old leaf bases are regularly occupied by groups of robust bracts, few in number and apparently surrounding the ends of slender old peduncles. The trunk may of course have died down. (An illustration is given on Plate LXX of U. S. Geological Survey Monograph XLVIII; the type is in the U. S. National Museum. It is the *Cycadeoidea Stantoni*. Thin sections are not available.)

2. The type of *Cycadeoidea nigra* from Boulder, Colorado, is a trunk segment of considerable size from the mid-region of a columnar stem supposedly a meter high. Again each leaf-base axil is occupied by an old, rather slender peduncle bearing few and heavy bracts. Thin sections have been cut from the armor, and should be amplified. No basal parts of fruits have been observed; but as in the preceding instance the fruiting space appears occupied, and a final series is indicated. The flowers may have failed of conservation, or the stem may be old. This type is receiving further study. It belongs to the Colorado School of Mines, and full citations and description may be found in Wieland, *American Fossil Cycads*, volumes I and II (Carnegie Institution of Washington Publication 34).

3. *Cycadeoidea Masseiana*, a remarkably fine columnar trunk segment greatly resembling the foregoing, from the "scaly clays" of the flanks of the Apennines, and reposing in the Capellini Museum, Bologna. Thin sections regularly cut young, small fruit axes in the axils of the large, old leaf bases of the very heavy armor. Again the thin sections should be much amplified. But as even the lesser ones cut by both Capellini and myself show the fruits, it is certain these are very numerous, and quite uniformly young. A fine model of this specimen is in the Yale collection of study materials. (Further reference in *American Fossil Cycads*.)

4. *Cycadeoidea Fisherae*, which may be only some varietal form of the *Cycadeoidea marylandica*, from the Potomac formation of Maryland, exhibits a most remarkable series of very young axillary fruits. No sections have ever been cut from this specimen, the conservation being hardly equal to that of either the Italian or the Black Hills trunks; though it is certain that nearly all the way from base to apex there is a fruit of some form in the axil of each leaf. It is of course possible that two floral forms are present, one staminate, the other ovulate; and it may here be remarked that, with

the accumulation of detailed studies of exceptionally silicified trunks, many interesting features and variations of cycadeoid fructification can yet be brought to light.

5. The most remarkable petrified plant of any kind ever recovered is no doubt the National Museum specimen found by Dr. Darton on the eastern Black Hills "rim," and illustrated by some ten plates in volume II of *American Fossil Cycads* as *Cycadeoidea Dartoni.* The type consists in the upper half of a columnar trunk perhaps a meter tall, with a finely conserved terminal bud. The armor is literally packed with hundreds of mature cones, with the dicotyledonous seeds conserved in great numbers. There are very few old or aborted axes of any kind, and, while near the summit there are a few smaller and perchance younger fruits, the great series is uniformly mature. Some further sections from about the apex remain to be made. But it is to be cited that the old leaf bases appear desiccated or shrunken, while ample sections show no trace of a succeeding foliar crown; though a fine crown of young fronds surmounts a scattered growth of flower buds in the very different species *Cycadeoidea ingens*—a great type of six hundred pounds' weight from the Piedmont-Black Hawk locality some twenty miles due north of the point where the *C. Dartoni* was found. Yet other species could here be cited.

In view, then, of comparable species with the full series of lateral axial fruits immature, as in *C. Masseiana* and *C. Fisherae,* and of further types with peduncle series, or with isolated fruits, the *Cycadeoidea Dartoni* appears monocarpic. Vegetative growth has apparently ceased in a time of culminant fructification with the emergence of all the axes the stem can ever produce. But it must be admitted that if only a small percentage of the apical fruits are found younger or less full grown than the greater lateral series, a partial monocarpy or pseudomonocarpy is indicated. Of this more, with extension of thin section series. The available series of large tandem sections is shown on Plates 46–50 of *American Fossil Cycads,* volume II, so that the reader may somewhat judge for himself. It may be added that the tandem sections of *C. Dartoni,* though not at any time regarded as complete without continuation to near the crown, traverse the trunk from the base of the recovered segment upward for 35 centimeters, cutting nearly fifty seed cones. And throughout this long distance a series of uniformly mature cones is present, and evidently extends near to the trunk summit. In the belt crossed by the sections there is an average superficial area of five to six square centimeters to the cone, indicating for this upper trunk segment not less than 500 cones with the ripe embryos. On the basal segment of the trunk, which was not recovered (and which I have twice sought for at the locality on Battle Creek), there were at the lowest estimate as many more, or 1,000 in all for the entire stem (Plate XI).

If a yearly cone production is concealed above, it had become small. Among so many cones, imperfectly grown examples are to be expected, and certainly a few of the topmost of the cones below the terminal bud appear greener and less mature. In one cone inserted 6 centimeters below the apex, the seed stem mass is split off from the receptacular cushion, with the lower portions of the stem bundles left behind adherent to the cushion, so that these bundles are seen to traverse the clear silica the full distance between the cushion and the seed stem mass. The seeds of this cone are not conserved, but the bundles show a somewhat similar tension or tearing out in occasional cones farther down in the series. In a smallish cone five centimeters below the terminal bud, the basal seed stem mass is again all that is conserved, but it appears mature. These two bases of cones may be seen in the photograph of the full longitudinal section of the entire trunk segment (Plate 43 of *American Fossil Cycads*, volume II).

Pseudomonocarpous Types

Apparently monocarpy is a phenomenon at present confined to the angiosperms. At least no characteristic instances now occur in the gnetaleans, and none in the conifers. The latter are no longer capable of annual or biennial fructification, as perchance in some remote period of their history. The dwarf or pygmic species are simply those of the high mountains, such being stubbornly coniferous and longevous.

It is therefore curious to find that there is in the cycadeoids a near approach and parallel to a kind of pseudomonocarpy found in several conifers of the California Sierras. The so-called "knob cone pine" (*Pinus attenuata*) occurs in much restricted "close willowy groves" of the dry slopes —in the San Bernardino range at about 3,000 feet, or along lower forest limits. It is lower and more abundant in the Shasta region. This pine varies greatly in size, from 30 meters high down to only two, though mature. (The wood is of low specific gravity, only 0.35.) At the age of seven or eight years the large cones begin to appear, borne close to the stem or larger branches, and there persist from year to year until the older series is quite imbedded in the bark. So indurated and resistant are the sporophylls in fact that often the seeds are not shed until the trees die, the groves being periodically fire swept. Then, the cones split open and reseed as if from a single crop, the trees in the "stands" always being of the same age.[3] A life-like picture of this singular pine is given by John Muir.

Also, *Pinus Coulteri*, as I noted in the San Bernardino range, bears while younger a certain resemblance to the "knob cone." The very large cones are then borne only on the excurrent stems, and the first cones may abort for some distance up the trunk, leaving near the summit only a few, or but a single pair, of the mature cones about oppositely borne. The basal scales of the cones may be quite grown round by the bark. As the tree is destined to grow forward, there is of course nothing like an initial stage of true monocarpy in which apical growth must fail.

---

[3] It is evident that the cones near the base of an old tree reached maturity while the stem diameter was yet small, and that if they remained in the position where they first grew, they must become imbedded in the solid stem wood. But what happens is that the quite sessile cone is torn from its seat and carried outward by stem growth, with the bases deep set in the bark. The long persistence of the cones is partly due to an uncommonly hard resinous lacquer-like coating.

Obviously in those species of Cycadeoidea such as *C. dacotensis* and *C. Reichenbachiana*, with a very heavy armor and scattering fruit axes, there may have been retention of the mature cones for several seasons, closely packed in between the leaf bases and there kept dry and free from decay by an abundant drooping ramentum. But the cones themselves did not tend to persist intact after maturity, because the Araucaria-like grouping of the sporophylls allowed easy separation and splitting free of seed stem and interseminal scale alike; while a simulated monocarpy does not sufficiently explain the condition found in the several cycadeoid species above cited. In order, however, to reach certain main conclusions it is necessary briefly to bring to view the xerophyllous character of the heavy-stemmed cycads, their geographic range, and also the climatic factor.

Xerophyllous Features of the Cycadeoids

Two main series of the petrified cycadeoid stems occur in the western Cretaceous. The lower series is from the Morrison (Como of Marsh). At one point near the so-called "Bone Camp" in the Freeze Out Hills twenty miles northerly from Medicine Bow, Wyoming, the trunks have been found abundant in close association with the sauropod dinosaurs; but the cycad species again recur more scatteringly with the dinosaurs in the western Black Hills "rim." The stems are so beset by scaly ramentum that resemblance to an "old man cactus" was early suggested, as some evidence of growth in dry situations, although this idea is neither confirmed nor contradicted by the presence of a fairly primitive pine (?) which I lately found closely associated with the cycads of the "Bone Camp" (so named from the bones of the great dinosaurs there found). The wood of these early pinaceans is dense, the wood rays are small, and the growth rings are very pronounced. Evidently growth was sharply seasonal, summer and winter, or dry and wet.

The second and greater series of petrified trunks occurs in the succeeding Lakota of the Black Hills, with several hundred feet of sediments intervening. The horizon is considerably younger, and conifer stems are again conspicuously associated, though insufficiently studied. One is an Araucarian, called by Knowlton *Araucarioxylon Hoppertoniae.* Again the growth rings are pronounced.[4]

---

[4] This wood offers a simple but strong structure contrast to that found with the cycadeoids in the Freeze Outs. That wood is less Araucaroid, although the stems are above spoken of as early Pinaceans or Abietineans, in only a broad or perhaps Jeffreyan sense. I do not find full identity with any described form, though a relation to Prepinus (Hollick and Jeffrey) may be indicated by an imbedded shoot with centripetal wood.

Whatever these conifers may mean, the cycads are in their entire organization highly xerophyllous. There is, first, the profuse scaly ramentum thickly investing the frond bases as far out as preserved, and found scant in only a few instances (*C. Stilwelli* of the Black Hills, and an undescribed form from a much higher horizon in Alberta). Also, as Dr. Stopes has very lately found in a young lateral leaf crown long since cut at the British Museum, the under surfaces of the pinnules bear densely packed hairs. The feature is present in the American leaf series but varies greatly in its development with the species, of which at least four with well defined hairy leaves are known. The dense packing of pinnule hairs in *Cycadeoidea ingens*, as faintly preserved, simulates a tissue, in some parts of the frond as thick as the folded pinnules, and ending in a clear, sharp chalcedony line. These hairs are well cutinized, and the reduced transpiring surface, taken with the latitude (44°), suggests a warm-temperate desert climate. That these features and facts were long overlooked is quite inexcusable, though partly explained by the intention to come back to the critical study and illustration of cycadeoid frond structure. The character may be expected in some of the Mesozoic imprints, and is in physiologic accord with the free growth of angular scaly tomentum borne by the cycadeoid microsporophylls as they form the domelike apex of the young flower buds.

The foliage crowns of five American species of cycadeoids are structurally known. These are in order of discovery: *Cycadeoidea ingens*, *Cycadella wyomingensis*, *Cycadeoidea colossalis*, *C. marshiana*, and *C. dartoni*. But many more trunks with crowns remain to section.

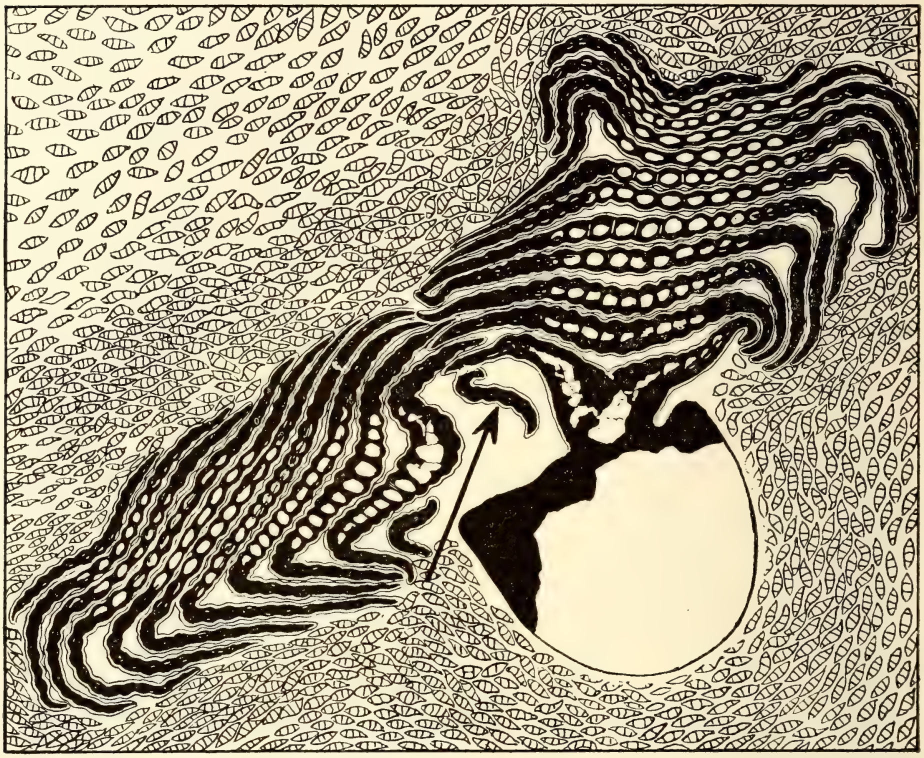
In general appearance the mature frond is absolutely determinate. The petiole was heavily clad in ramentum, varying considerably with the species, and becoming hairy instead of scaly about the insertion of the lowermost pinnules. Thence upward the upper surface of the rachis continues hairy with the hairy growth running out more or less freely all over the under surface of the pinnules; while the lower rachial surface and upper face of the pinnules remain smooth. (Note Plate XII, and text fig. 1; also Plate X, showing comparable Jurassic imprints.)

The picture, therefore, of the heavy-stemmed cycadeoids is a very well defined one of dry desert plants with all their parts, stem, fronds, and fruits beset by scales, hairs, or tomentum. And, moreover, a curious but deeply interesting bit of confirmatory geologic evidence is afforded by the presence in the Lakota of reefs of calcareous tufa accompanied by numerous polished pebbles, held to mark recedent lake shores of increasingly dry regions. Though it would be an error to view the cycadeoids as dependent on as

There are no resin canals, and no bars of Sanio or other modern features in either the tracheids or the rays. The radial tracheid pits are in one row, very rarely two, and never crowded or compressed. The rays are one to twenty cells high, normally ten, and one cell thick,—or two, for a distance of a cell or two.

It is probable that both these types of wood, Araucaroid and Pinoid or more Abietinoid, will yet be found occurring alike in both of the cycadeoid horizons.

distinctly tropic climates as present-day cycads. In accord with a world-wide distribution, a distribution exceeding that of cycads all the way from Florida to near the pole, the former had plasticity of both flower and leaf. Whether to be viewed as forerunners of the angiosperms or not, the capacity to live in varied climates must be hypothesized. It is not, however, likely that the Dakota cycadeoids, fringing as they did more or less pure-stand forests of pines and araucarians, could have endured as severe a climate as that of the Argentine "stands" of *Araucaria imbricata* facing the dry treeless country to the west of the Patagonia plateau in south latitude 35°–38°. But with the young leafy crowns so thickly beset by ramentum and the fruits so enclosed by bracts and the armor, there was full protection from many degrees of frost and from a time of snow.



TEXT FIG. 1. *Cycadeoidea Dartoni* (?). Young frond deeply imbedded in the petiolar ramentum. The arrow shows the basal ears as in Otozamites. The two ranks of folded pinnules face the trunk axis. Finer hairs and histologic structure not conserved. Transverse thin section × 30.

RANGE OF LARGE-STEMMED CYCADEOIDS

In North America, on the Atlantic-Gulf border, the evidence of an extended cycadeoid habitat is conclusive. Low down in the Potomac formation of Maryland in the Arundel (?) occurs the characteristic *Cycadeoidea marylandica* in latitude 39°. And twelve hundred miles to the southwest this same form recurs in Wise County, Texas, latitude 33°.[5]

About the same time the Arundel cycadeoids formed this southeastern continental fringe, a second and highly xerophyllous group of very small trunks finds a certain extension from the Freeze Out Hills of Wyoming to the Black Hills, or from latitude 42° to 44°.

As the greatest of all American occurrences, the cycadeoids of the succeeding Lakota girdle the Black Hills in latitude 43° to 45°, while two isolated finds, which may be nearly associated, extend this range on from Colorado to California on the 39th parallel, about 1,000 miles.

Correspondent to this greater North American extension, there is in Europe a triangle of occurrence with an apex in the Apennines in latitude 44°, and with a base of 1,000 miles from the Galician Carpathians and Cracow in latitude 50° to the Isle of Portland in latitude 51°. As in America, the north-south limits are only seven degrees apart. But high in the upper Cretaceous these limits are slightly extended. In the Upson Shales of Maverick County, Texas, occurs the isolated *Cycadeoidea Uddeni* in latitude 29°, while in the Belly River formation of Alberta, of nearly the same age, an undescribed cycadeoid is found at the north limit, latitude 54°. Whether this represents actual late extension of habitat cannot be said.

Finally, the petrified cycadeoids of India come about on the Tropic of Cancer. Whence, unless the exigencies of silicification be so great that most of the record of the heavy-stemmed cycadeoids must forever lie hidden behind the thick veil of the paleontologic past, restricted habitats are inferable, and those always with a large part of the year decidedly dry. That the cycadeoid alliance as a whole was of cosmopolitan distribution all through the Mesozoic should indicate the further capacity to live in the most varied climates, as already maintained. But that which it is especially desired to point out is that the greater silicified series of early Cretaceous time falls at a period of considerable continental emergence, in North America at least, and goes far to indicate long arid belts there covering the 35th–45th parallels, in Europe the 45th–51st. Elsewhere attention has been called to the fact that the record for Asia and Africa, likewise for South America and Australia, fails.

---

[5] The Texas cycadeoid here referred to is so like the original Maryland type that it would be regarded as varietal if from the same locality. It is an isolated and thus remarkable find of Dr. Emilio Böse with whom I was associated as a paleontologist of the Instituto Geologico of Mexico during the years 1908–10. With great consideration Dr. Böse apprised me of his "find" the day it was made, in July, 1915, and later sent me the specimen. It is from the basal Trinity Beds. These rest on the Pennsylvanian, and are generally regarded as not the very oldest Cretaceous in the broader sense, but about the age of the European Aptian.

Early Cretaceous Climate of the Cycadeoid Habitat

In no case is it necessary to assume a Saharan heat, or even a markedly warm temperate climate, for the North American cycadeoid belt. As above pointed out, Cycadeoidea had the habitus to resist dry cold, while the associated conifer forests might well have been as able to withstand zero temperatures [–15° to –20° C.] as the Chilo-Argentine *Araucaria imbricata* forest with its lower limit undergrowth of monocarpic bamboos. Nor is it improbable that a closer scanning of the contemporaneous floras of the Lower Cretaceous "Rim" of the Black Hills as recorded from both the Morrison and Lakota, and yet destined to great extension, will compel much revision of our views of *all-tropic* Mesozoic climate. Even a brief notice of what is already known here may be illuminating.

In the 19th Annual Report of the U. S. Geological Survey, Fontaine gives an account of the plants below the Dakota—that is from the Lower Cretaceous "Rim" of the Black Hills, immediately in, above, or slightly below the great series of cycadeoids from Minnekahta and the Piedmont-Black Hawk localities. Of dicotyls there are four, called Ulmiphyllum, Ficophyllum, Quercophyllum, and Sapindopsis. Two of these occur in the Potomac of Maryland, and all are small-leaf types. It is only by a kind of elaborate argument carrying these generalized types, only known within family limits, into other zones, that they can be made to appear to have lived solely in tropic climates. And the same is true of a few species each of more or less closely associated ginkgos, conifers, and araucaroids—all small-leaf species that by themselves would easily fit into dry, to even cool climates. While three small-leaf plants of presumably cycadeoid affinity find their nearest relatives in Oregon and Greenland. Once more it is only by cumulation of disconnected inferences based on series which require much further study that these plants can be brought into the *all-tropic* scheme. Of the fern assemblage—all small of leaf—little more is known than that according to previous interpretation they easily fit the tropic category because resembling their Jurassic antecedents.

It is early to attempt a revised, well founded estimate of the full climatic significance of the Lakotan plants. This would in fact involve a further study of all Lower Cretaceous floras. Though it is evident that too much weight has been given to the superficial resemblances in extinct floras. These must always have a certain prevailing cast due to the average course of geologic change continental in magnitude. True, if a widespread tropic plant facies marks the early Mesozoic, the identical or closely related species must have a wider north-south range than later on; *but always, the similar elements of given floras, which are a reasonably sure indication of synchroneity, are only the unsafe criteria of climatic equivalence.*

A General Conclusion

On venturing beyond the simpler comparisons with nearly related fossil types, it becomes difficult to discuss such a highly specialized and xerophyllous plant as Cycadeoidea. There is the immense pith, the persistent armor, and the cauliflorous habit, or production of fruits along the stem below the foliage crown. The great thickness of cortical parenchyma in some forms need not be mentioned, since in some of the types with a quite heavy woody cylinder, as heavy at least as in the Cordaiteans, the pith is somewhat reduced, the cortex markedly so.

But, in the *first place*, Cycadeoidea in actuality nears certain conifers of robust form, and, where branched, finds some resemblance in Araucaria. In the tallest cycadeoid trunks, the height may be at least six times the stem diameter, exclusive of the armor of leaf bases. Now, in the Chilean *Araucaria imbricata* forest, normally grown old trees may be found with a height of only ten or twelve times the diameter of from three to six feet, while in the Sequoias of the Mariposa grove a like ratio is found in the case of various of the larger trees. For the cypress, taking the gigantic *Taxodium mucronatum* of southern Mexico, the ratio is little if any higher than in Cycadeoidea, in contrast to fifty or sixty in the taller conifers.

Secondly, the wood of *Cycadeoidea* is of a generalized type which would permit innumerable derivatives. The comparative study of tracheids suggests this, and it must be true if the complex wood ray structures of gymnosperms and angiosperms are mainly of Mesozoic origin, as the paleontologic record appears to indicate.

Thirdly, there is again full sanction in the Mesozoic record for hypothesizing progressive pith reduction all through that age, not merely in cycadeoids, but in gymnosperms generally. And with pith reduction, branching would set in, with great floral and reproductive, as well as foliar, change; though here a reverse process may also be conceived, dependent on habitat as tropic or high northern.

Fourthly, the cycadeoid floral type permitted all the sex variation possible in any flowers. The angiosperms can vary no more.

Fifthly, monocarpy which is subject to certain variations in existing plants is, in a modified, possibly in a true form, present in the cycadeoids. Great lateral series of young fruits, as well as old peduncles seen in four species afford the main evidence.

Sixthly, the cycadeoids of the silicified series are so strikingly xerophyllous that they go far to indicate vast dry to even cool belts in the mid-temperate to northern temperate zone in both Europe and America. The organization and protective features are such that light snows and some degrees of frost could have been withstood.

Seeing, therefore, that there can have been such vast change and variation in the more generalized cycadeoids, it becomes evident that there could also be notable changes in flowering as related to length of life. Hence, even to establish the presence of a pseudomonocarpy is of deep interest. If a raceme were to lengthen out its life it would become pseudomonocarpic. Evidently both mono- and pseudomonocarpy must be capable of variation with change in the length of life. And both, though in a different way, are either accompaniments of gigantism or the essential characteristic of communal forms.

EXPLANATION OF PLATES

Plate IX

Cycadeoidea superba. A southern Black Hills trunk with five large, low subglobular branches, 75 centimeters across. Various sparsely distributed flower buds are noted, and it is quite certain that the main fruit production would have occurred later had not the initial event leading to fossilization intervened. The crowns have never been examined for the young fronds, although this form of Cycadeoidea compares most closely with the highly xerophyllous Encephalartos.

Plate X

Portion of a slab from the Lias of the Barranca Consuelo, Oaxaca, Southern Mexico, bearing three species of Cycadeoids (?): *Otozamites Reglei* (the three complete fronds above), *Otozamites Juarezii* (large single pinnule), and *Otozamites hespera* (lower frond with narrow pinnules). Latitude 17°. These plants with the flora associated may be taken to indicate a Mexican Liassic climate much like that of today, with a probability of somewhat drier, or even of desert and cooler conditions. Slightly reduced.

Plate XI

Cycadeoidea Dartoni. Portions of large sections traversing the many cones with the mature seeds. The dicotyledonous embryos quite fill the seed cavity, and the narrow slit separating the two cotyledons may be detected in many instances. Fig. 1 from a thin section. Fig. 2 from a polished surface. Enlarged.

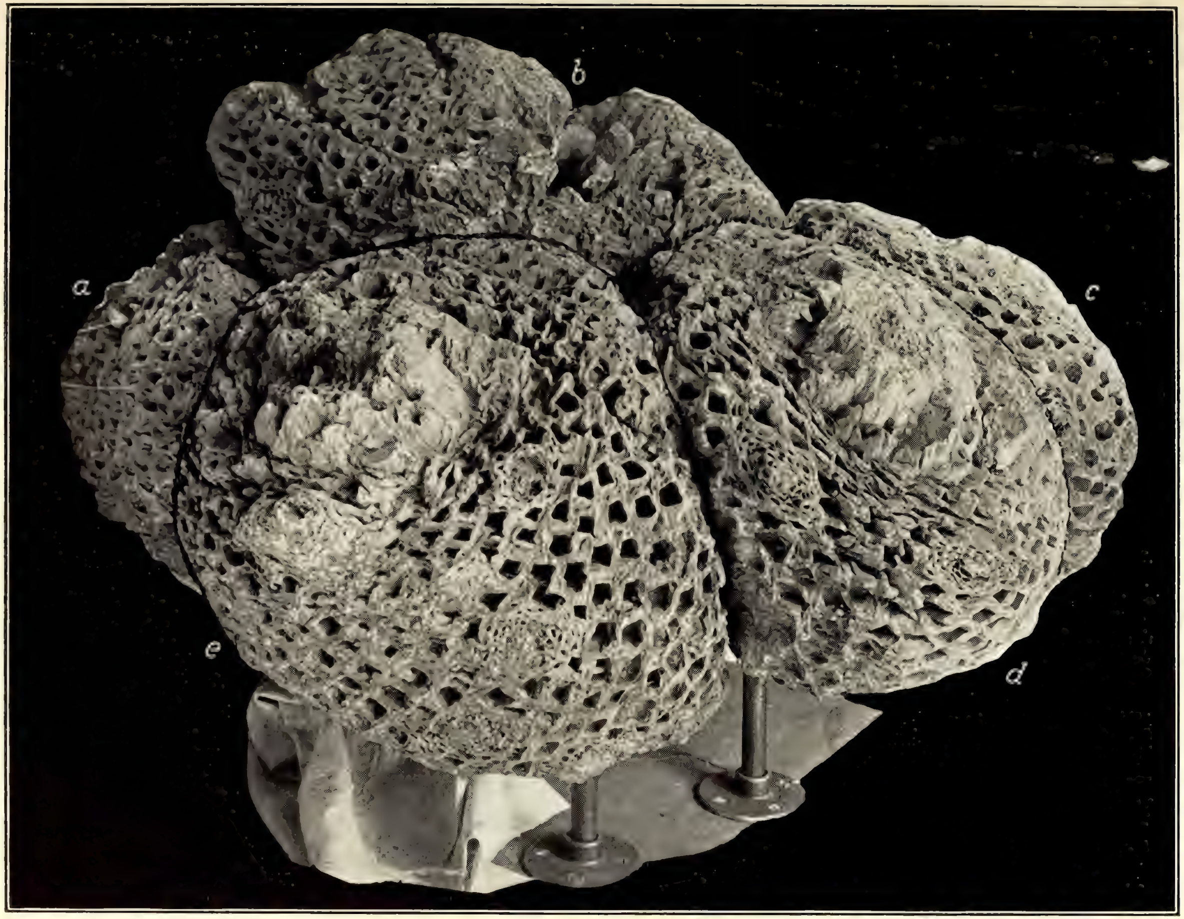
Plate XII

Fig. 1. Transverse section through the bisporangiate strobilus of *Cycadeoidea dacotensis* showing young central cone, staminate disc (with decurved frond tips), and outer bract husk. Enlarged.

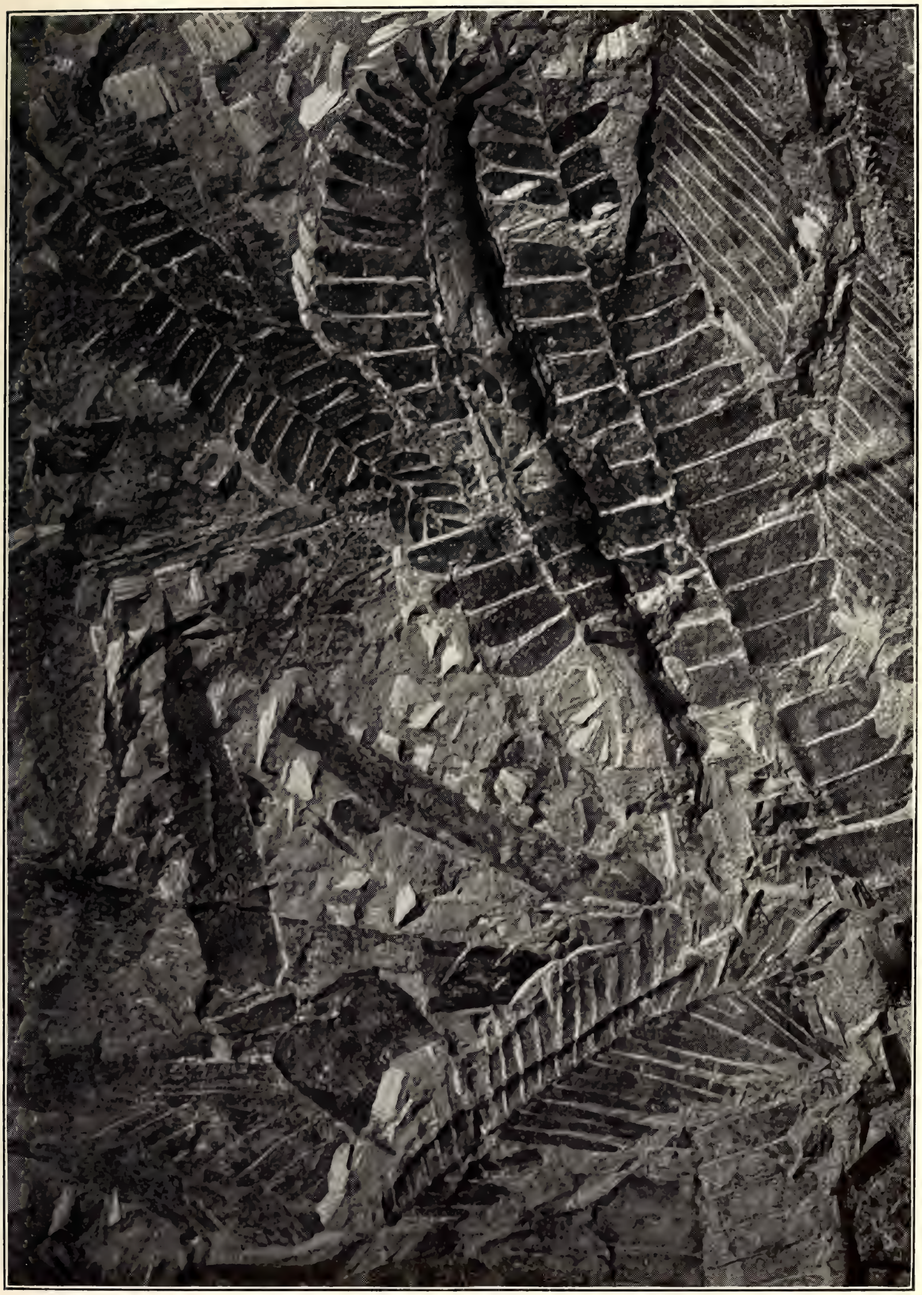
Fig. 2. *Cycadeoidea ingens.* Young non-emergent frond with folded pinnules. The most heavily haired frond type. The light dotted line of tissue is the pinnule, with a shaded band nearly as thick indicating the packed hairs. Transverse × 3.

Fig. 3. *Cycadeoidea dacotensis.* Young folded frond of the lightly haired type, but with the bases of the cutinized hairs beautifully conserved, though not showing well under about fifty diameters. Transverse × 3.

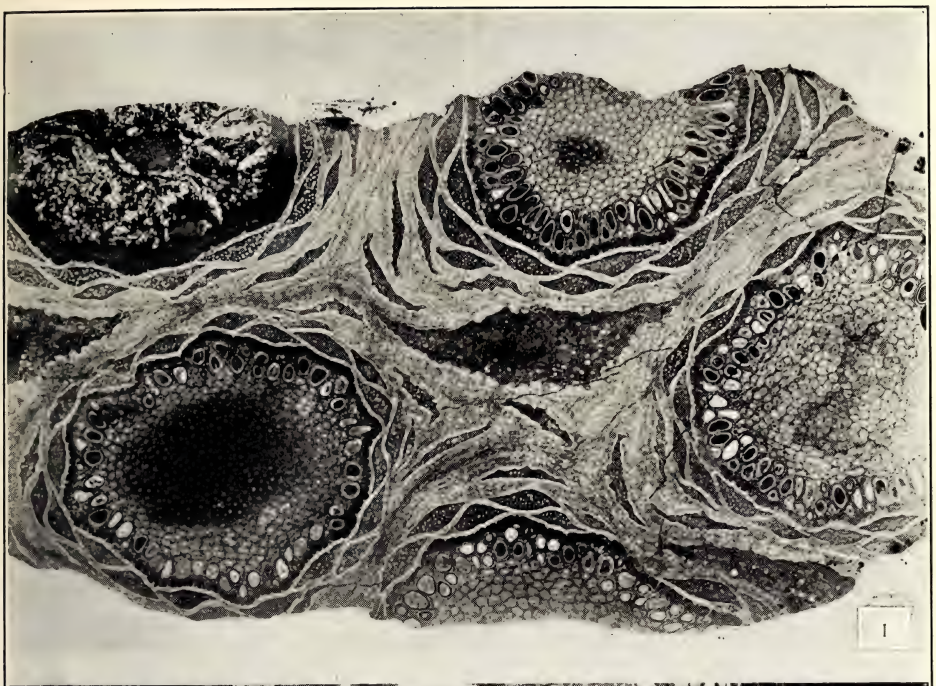
Fig. 4. *Cycadeoidea ingens.* Young folded frond with slight development of the haired feature of the pinnules. Transverse × 3.



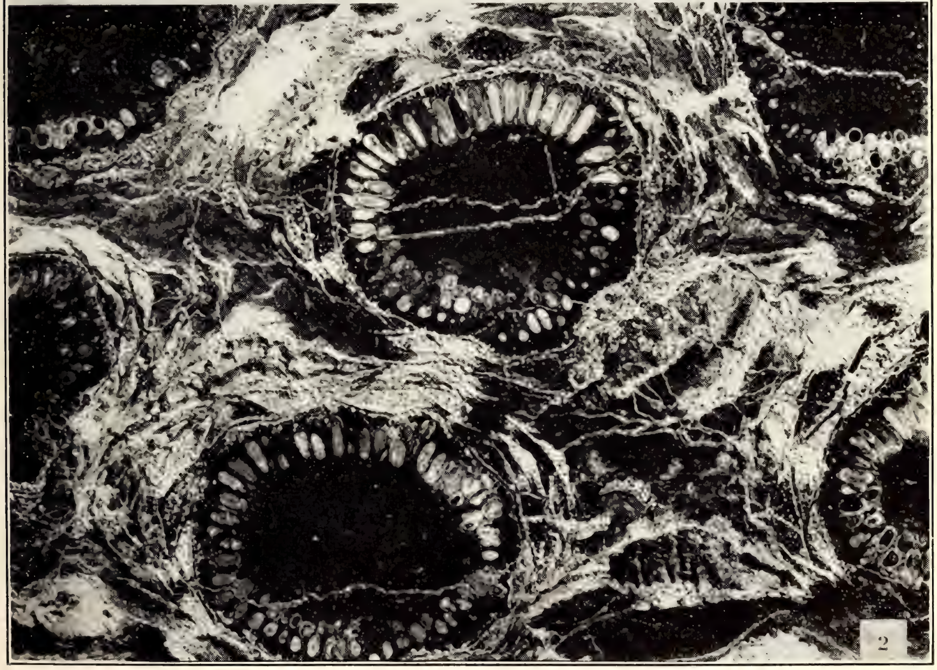
WIELAND: CYCADEOIDS.



WIELAND: CYCADEOIDS.

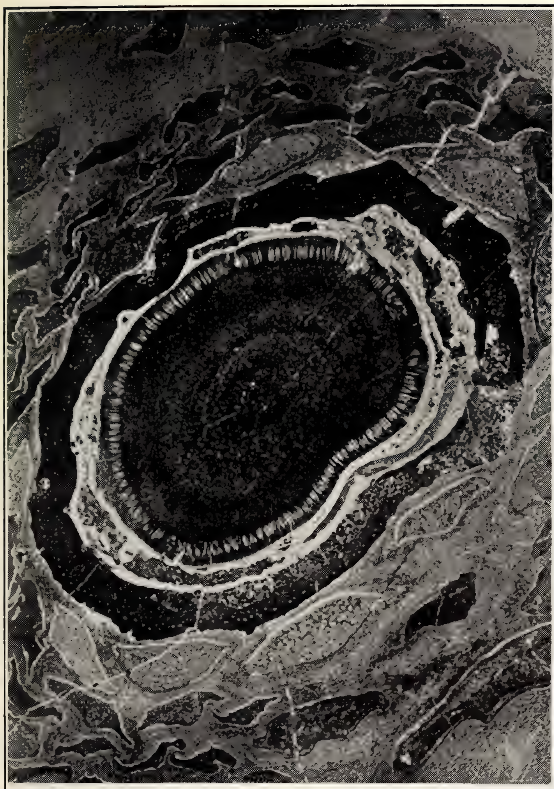


1

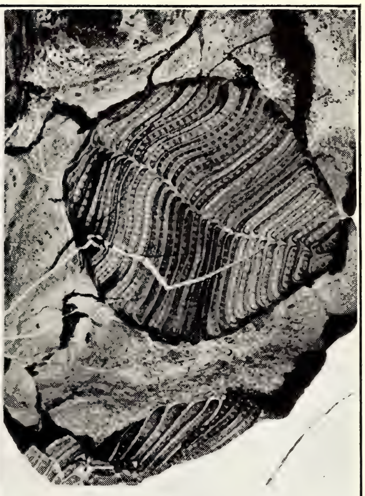


2

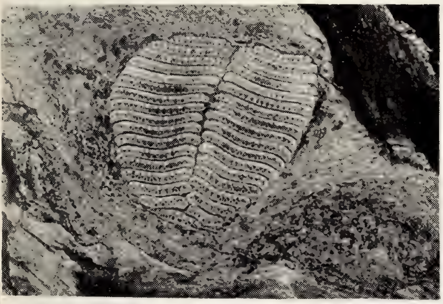
WIELAND: CYCADEOIDS.



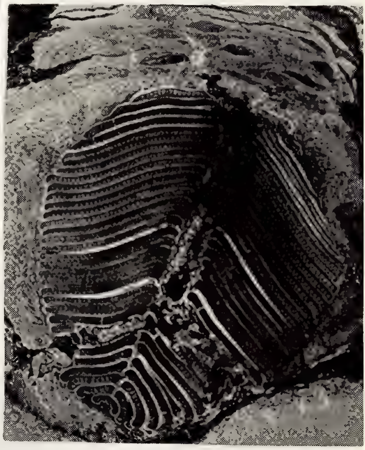
1



2



3



4

WIELAND: CYCADEOIDS.

Harvard Summer School

JULY 5 to AUGUST 13

1921

Courses in Elementary Botany, Morphology, and Plant Anatomy are offered by the Harvard Summer School for 1921. A limited number of research students can be accommodated in Botany S20a. Correspondence in regard to the various courses should be directed to Professor E. C. JEFFREY, 47 Lakeview Avenue, Cambridge, Mass., until the 5th of June. From that date until the opening of the School, inquiries should be addressed to Dr. R. E. TORREY, Department of Botany, Massachusetts Agricultural College, Amherst, Mass. Requests for information may also be sent to the Director of the Harvard Summer School, 19 University Hall, Cambridge, Mass.

UNIVERSITY OF COLORADO

SUMMER QUARTER

Location: Boulder, Colorado, at the mountain front of the Rockies; environment favorable for study, for field work, and for outdoor recreation. Easy access to all of the life zones from plains to alpine heights and glaciers.

Courses in Botany and Zoology: College Botany, Economic Botany, Bacteriology, Botany of Ornamental Plants, Ecology, Local Flora, Forest Botany, College Zoology, Nature Study, Ornithology, Economic Zoology, Cytology, Heredity.

Other Courses in all usual academic subjects.

Summer quarter of two terms: June 13 to July 21 and July 21 to August 27.

Catalogue may be obtained by addressing the Registrar of the University, Boulder, Colorado. For any special information regarding courses in botany and zoology address Professor Francis Ramaley, University of Colorado, Boulder, Colo.

Brooklyn Institute of Arts and Sciences

The Biological Laboratory

At Cold Spring Harbor
Long Island, New York

32d Season. June to September, 1921.

Courses, July 6—Aug. 16

Courses are offered in field zoology by Drs. H. E. Walter, S. I. Kornhauser, and H. M. Parshley. Comparative anatomy, Dr. H. S. Pratt. Systematic and field botany, Drs. O. E. Jennings, John W. Harshberger and C. A. Stiteler. Genetics by Prof. H. D. Fish. Opportunities for beginning investigation under the direction of any of the above instructors. Facilities offered for independent investigation, without fee.

Tuition in all courses, $30. Further details are given in the announcement of the Laboratory, for which address the Biological Laboratory, Cold Spring Harbor, Long Island, N. Y.

THE ADIRONDACK SUMMER CAMP

OF THE

New York State College of Forestry

AT SYRACUSE UNIVERSITY

June 6 to September 3, 1921

Location: On Cranberry Lake, in the heart of the Adirondack forest.

Field Courses in Plant and Animal Ecology, Mycology and Forest Pathology, Dendrology, Forest Entomology and Silviculture.

Students may register for any or all courses.

Investigators will be welcomed and all facilities of the camp will be placed at their disposal.

General Courses in Botany and Forestry are offered in the Summer Session of Syracuse University, July 5 to August 12, 1921.

For further information, address the **Director of the Summer Session,** Syracuse University, or **Director of the Summer Camp,** N. Y. State College of Forestry, Syracuse, N. Y.

The Little Things That Count Big In Owning Your Own Greenhouse

Ever gone into a hat store, knowing exactly the kind and shaped hat you want; and then have some smart Alec of a salesman try to sell you the kind HE likes?

Makes a fellow madder than a wet hen, doesn't it?

Suppose, however, that same salesman had told you something about some of his hats that meant better service to you—more satisfaction for your money—you then sort of feel towards that salesman as you would an old friend.

Salesman number two is our sort when it comes to selling a greenhouse.

We do sell greenhouses, but we first sell satisfaction.

Catalog for the asking.

Hitchings and Company

Home Office and Factory: ELIZABETH, N. J.

NEW YORK
101 Park Ave.

BOSTON-9
294 Washington St.

Vol. VIII. MAY, 1921 No. 5

AMERICAN JOURNAL OF BOTANY

OFFICIAL PUBLICATION OF THE
BOTANICAL SOCIETY OF AMERICA

Smithsonian Institution
MAY 24 1921
National Museum

CONTENTS

Isoachlya, a new genus of the Saprolegniaceae . . . . . C. H. KAUFFMAN 231
The transmission of Rhus poison from plant to person . . JAMES B. MCNAIR 238
Ths type concept in systematic botany . . . . A. S. HITCHCOCK 251
The relation of certain nutritive elements to the composition of the oat plant
JAMES GEERE DICKSON 256

PUBLISHED
IN COOPERATION WITH THE BOTANICAL SOCIETY OF AMERICA
BY THE
BROOKLYN BOTANIC GARDEN
AT 41 NORTH QUEEN STREET, LANCASTER, PA

Entered as second-class matter February 21, 1914, at the post office at Lancaster, Pennsylvania,
under the act of March 3, 1879

AMERICAN
JOURNAL OF BOTANY

DEVOTED TO ALL BRANCHES OF BOTANICAL SCIENCE

ESTABLISHED 1914

EDITED BY A COMMITTEE OF THE
BOTANICAL SOCIETY OF AMERICA

EDITORIAL COMMITTEE

C. E. ALLEN, Editor-in-Chief,
University of Wisconsin

WILLIAM CROKER,
University of Chicago

L. R. JONES,
University of Wisconsin

C. STUART GAGER, Business Manager
Brooklyn Botanic Garden

ORLAND E. WHITE,
Brooklyn Botanic Garden

R. A. HARPER,
Columbia University

JACOB R. SCHRAMM,
Cornell University

C. L. SHEAR, *Bureau of Plant Industry*
(Representing American Phytopathological Society)

The Journal is published monthly, except during August and September. Subscription price, $6.00 a year. Single copies 75 cents. Back numbers, 75 cents each; $7.00 a volume. Postage will be charged to all foreign countries, except Mexico, Cuba, Porto Rico, Panama Canal Zone, Republic of Panama, Hawaii, Philippine Islands, Guam, Samoan Islands, and Shanghai. Postage to Canada, 20 cents a volume on annual subscriptions; to all other countries in the Postal Union, 40 cents a volume on annual subscriptions.

The pages of the Journal are open to members of the Botanical Society of America, or to candidates approved for membership.

Manuscript offered for publication should be typewritten, and should in all cases be submitted to the Editor-in-Chief.

Papers are limited to 20 pages in length, exclusive of plates, except that additional pages may be arranged for at cost rate to authors (approximately $3.00 a page).

Proofs should be corrected immediately on receipt, and returned to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y.

Illustrations, other than zinc etchings, and tabular matter should, in general, be restricted to approximately ten percent of the number of pages of text. Authors may be charged at cost rates for illustrations and tabular matter in excess of that amount (approximately $6.40 a page for tabular matter; 15 cents per square inch for half-tones).

Separates should be ordered when proof is returned. Ffty copies without cover will be supplied free; cover and additional copies at cost.

Remittances should be made payable to American Journal of Botany. Ten cents must be added to all checks not drawn on New York City banks.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

Correspondence concerning editorial matters should be addressed to Prof. C. E. Allen, University of Wisconsin, Madison, Wisconsin.

Business correspondence, including notice of change of address, and directions concerning reprints, should be addressed to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y., or 41 North Queen Street, Lancaster, Pa.

AMERICAN
JOURNAL OF BOTANY

VOL. VIII MAY, 1921 NO. 5

ISOACHLYA, A NEW GENUS OF THE SAPROLEGNIACEAE[1]

C. H. KAUFFMAN

(Received for publication December 29, 1920)

Isoachlya Kauffman gen. nov. *Hyphae* rather stout or slender. *Zoosporangia* formed from their tips, oval, pyriform, ventricose-clavate, the later ones (secondary) arising either by cymose or pseudo-cymose arrangement as in Achlya, or by internal proliferation as in Saprolegnia, both modes occuring earlier or later in the development of one and the same species, or frequently on the same main hypha. *Zoospores* diplanetic, as in Saprolegnia, escaping and swarming separately, and after encystment swarming the second time before the formation of a germ tube. *Oogonia* terminal or torulose, occasionally intercalary. *Oospores* with centric contents, the spores filling the oogonium incompletely. *Antheridia* present or few to none.

The genus is characterized and distinguished, in the main, by the presence of the cymose or Achlya mode of formation of secondary sporangia, coupled with diplanetic zoospores. The following species naturally fall within its boundaries:

1. **Isoachlya toruloides** Kauffman and Coker sp. nov.
2. **Isoachlya paradoxa** (Coker) comb. nov. *Achlya paradoxa* Coker. Mycologia **6**: 285. 1914.
3. **Isoachlya monilifera** (de Bary) comb. nov. *Saprolegnia monilifera* de Bary. Bot. Zeit. **16**: 629. 1888.

Isoachlya toruloides Kauffman and Coker sp. nov.

Hyphae rather slender and short, 18–20 μ in diameter, later ones frequently smaller, straight and scarcely branched. Zoosporangia oval, pyriform, clavate-pyriform, more rarely elongated-pyriform, with a more or less distinct papilla; secondary sporangia, during the early and vigorous development, all cymosely arranged by successive basipetal formation, sometimes from the walls of earlier ones, later secondary sporangial initials appearing by internal proliferation as in Saprolegnia; zoospores diplanetic, capable of escaping and swarming separately, encysting after coming to

---

[1] After this paper was in the hands of the editor, a letter from Prof. W. G. Coker, of Chapel Hill, N. C., indicated that he was describing the same new genus and species. An exchange of data confirmed this supposition and hence it was agreed to publish them under joint authorship. The descriptions and figures to be given by Professor Coker have been examined by me, and I believe they apply to the same fungus.

[The **Journal** for April (**8**: 179–230) was issued April 30, 1921.]

rest, and after their second escape germinating by a germ tube. Oogonia globular, short-pyriform or pyriform-globular, with a more or less short hyphal base, produced at the ends of main hyphae in basipetal series, or mixed with the sporangia in a sporangial series, up to 6 in a series (as far as observed), persisting in series until or after maturity of oospores; their walls firm, soon becoming pale brown, with more or less scattered, distinct, small pits, sometimes numerous. Oospores centric, 1–3 in an oogonium, rarely 4 or 6, at maturity with a thick wall and a granular central sphere separated from the wall by a layer of uniform thickness; the spores measure 16–22 μ. Antheridia and antheridial branches none or very rare.

Two collections: in shallow water over peat-like organic remains, shore of First Sister Lake, Ann Arbor, Michigan, and in a pool of sphagnum near by. Taken November 23. Cultivated on the house-fly, *Musca domestica*, from single zoospores.

This species has for its nearest relative "*Saprolegnia monilifera* de Bary," from which it differs in that its oogonia are persistent in the series in which they are formed until after the maturity of the oospores and the breaking down of the supporting hyphae. The oogonial pits, although scattered, are distinct, while in "*Saprolegnia monilifera*" they are said to be few or none. In de Bary's species the most general mode of proliferation of the secondary sporangia is by the method found in the species of Saprolegnia, while in the present species they are formed for the most part by the cymose method, and for a considerable time during my observations of fly cultures I did not see the other arrangement, not indeed until the cultures became comparatively old. The appearance of the sporangia-bearing hyphae, and the mode of proliferation of successive secondary sporangia, can be seen by referring to figure 4, *a–c* (Plate XIII). This is the regular process on the house-fly in 30–40 cc. of water, varying only in minor details. The oospores in the oogonia are also less numerous than appears to be the case in de Bary's species, but the character may vary somewhat in this group, even on the same substratum—although within limits—by reason of slight differences in the vigor of the mycelium as a result of a larger immediate food supply. In "*Saprolegnia monilifera*" the oogonia are said to be produced in great abundance, and sporangial formation is reported to be almost completely ended when the oogonia begin to form. In *Isoachlya toruloides*, on the other hand, sporangia and oogonia are often formed at the same time, from the time oogonia begin to form until the age of the culture and the toxic condition of the water begin to produce an effect and chlamydospores appear instead.

The zoosporangia develop in the usual manner, and spore formation as seen in a living culture indicated nothing unusual. When the zoospores are ready to escape, the papilla at the apex of the sporangium is dissolved and they swim out at a fairly rapid rate and slowly scatter to a short distance before rounding up. I have followed this procedure in normal cases. A typical case is as follows. The culture had been kept in a cool glass-surrounded enclosure, to which a partially opened window gave access to outside temperature during the winter months. The temperature usually fluctuated between 6° and 10° C. The culture was four days old and had developed at these lower temperatures, and when examined on February 14 had been in a temperature slightly below 8° C. for at least twenty-four hours. Zoosporangia were very abundant, all primary and apical, many mature, several empty, and with motile zoospores present in the upper layer of water. These primary zoosporangia were pyriform to ventricose-pyriform in shape. In the course of half an hour or so, during examination in the warmer temperature of the laboratory, many mature sporangia opened, and in a relatively short time the water was alive with zoospores. There was every indication that they were already ciliated in the sporangium. As they lined up more or less within the sporangium, after the latter had opened, they assumed an oblong shape, obscurely curved on one side and with rounded ends, and not at all pyriform as frequently described for Saprolegnias; but, as was seen later during their evolutions immediately after escaping, they had an unequal diameter in their short axes, *i.e.*, they were slightly flattened.

A zoospore was uninterruptedly followed from its emergence, the instant it had become free of the opening at the tip of the zoosporangium. It was seen to turn over and over on its long axis, at the same time slowly getting away from the neighborhood of the others, which were all turning somersaults in the same curious way. Meanwhile the elongated, sub-oblong or slightly sub-reniform shape was retained. This continued for about twenty minutes, during which time every movement could be followed. Neither this spore nor the others, as far as observed, ever exhibited the darting or rapidly swimming habit of zoospores of the species of Saprolegnia. At the end of this period other manifestations began to appear. The spore—always turning—began in a more or less spasmodic manner to hump itself somewhat on its convex side, then to straighten out again, and after several such performances to show signs of a shortening process. Gradually during the last five minutes, by a twisting and shrugging process, it became much shorter and one end became contracted, so that a short pyriform shape, or an oval form with a papilla, resulted; this was soon changed, however, to a more and more globular form until finally a perfect sphere resulted. No let-up of the turning which it had exhibited over its longer axis could be detected up to this time, and even for perhaps twenty seconds after it appeared to have become perfectly rounded it still continued to revolve. Then very quickly all motion ceased, and the spore assumed its first resting condition.

Observations following this at intervals during the next few days showed that the spores, after rounding up, gradually sink to the bottom of the dish except when, sometimes in great numbers, they are caught in the mesh of mycelium surrounding the fly. After several days, the bottom of the dish is literally covered by a thin layer of such spores and an equal number of empty spore-capsules. In some cases the contents of the spores were seen emerging, but so slow did this process appear to be that none were ever observed to swim away in the second swarming stage. On the other hand, a great many spores whose structure indicated that they were the end product of the first swarming stage were seen germinating by germ tubes, which eventually became slightly branched by short outgrowths. This is not uncommon in other species of the family. By transferring these empty capsules along with germinated and ungerminated spores to a slide and adding chlor-zinc iodide, no immediate reaction occurred, although a mature oogonium in the mount took a deep stain. On adding to this a rather strong iodine solution (in potassium iodide), the characteristic color for fungus cellulose appeared. The walls of the empty capsules, of the spores, and of the germinating threads, were definitely stained. The spores, during the period after the first swarming stage until they germinated, became more and more vacuolate. The scanty food supply demands that the germination tubes draw upon the protoplasmic content of the spore, and eventually doubtless they will perish, thread and spore. Although several weeks had elapsed since the first spores had germinated, no sign of sporangial formation at the end of the germination filaments was ever noted, although this occurs frequently in other species when food supply is lacking in the solution, and is perhaps to be found as a specific characteristic of certain species under other conditions.

We may now turn to the reactions of this species under certain definite conditions. The description so far is that of its "normal" behavior on a sterile house-fly in sterile conductivity water of high purity. If grown on fruit-flies (Drosophila), the rapidity with which the stages of its development follow one another is quite marked. The sporangia appear many hours earlier after exposure of the flies to the zoospores, and sexual reproduction is several days ahead of the time for cultures on the house-fly. The food is more rapidly diffused and exhausted.

In a solution of haemoglobin 0.05 percent + KNO_3 0.1 percent, four days old, at the temperature mentioned before, the culture is found to have developed very abundant oogonial initials, arranged in torulose series as shown in figure 10, *a*, *b* (Plate XIV), and in some cases the terminal oogonium has already formed oospheres. The latter tend to be more numerous in each oogonium than is the case in fly cultures. At this same time the house-fly was densely covered with short, straight hyphae, nearly all bearing sporangia, mature or maturing or with sporangial initials. After ten days in this solution the earlier series had produced oospheres and some oospores, but those developed later showed signs of abnormality and disintegration.

In a solution (always with conductivity water) of haemoglobin 0.05 percent, in this case with an addition of 0.12 percent levulose, a culture four days old, under the same temperature conditions, showed the same general abundance of oogonial initials, but in this case the oogonial swellings occurred at the ends of stalks in pairs, in series of two, or with one on an adjacent short lateral stalk. After ten days the greater number of these had developed further, so that the terminal one possessed oospheres or oospores while the basal ones were initials and in many cases showed signs of never being able to complete their destiny. Later observations showed this surmise to have been correct, the apical ones maturing further, the contents of the basal ones breaking down. (See fig. 11, *a–c.*)

In a 0.1 percent solution of leucin, under the same conditions, after four days only scattered and quite young oogonial initials were seen. After ten days they were abundant, and a large proportion contained oospheres. But in this case the globular oogonia were borne singly at the ends of long supporting hyphae, not more than 1 to 2 percent being in series of two. (See fig. 12, *a–c.*)

In all the cultures mentioned, no antheridia were ever observed. The pits of the oogonial wall were always rather far apart, not large, and in leucin unusually distinct. The torulose habit of the oogonia is reduced as we go down this list of cultures. The presence or absence of some substance in the synthetic solutions is doubtless responsible for the inability of the development of the later oogonia to run its usual course. Although no combinations with mineral salts were extensively tried with the haemoglobin and leucin solutions, work on other species has shown that there is no reason to suppose that the disintegrating effects of the haemoglobin and leucin could not be offset by the addition of proper amounts of certain salts, the oospheres thus being induced to complete their development to maturity. It will be noted that the period of time necessary for the appearance of the reproductive organs is not strictly an inherent quality within the species, but depends, within limits, on certain other influences. The older investigations laid great stress on the exact period of time which any process, in the life history of a species, took to complete itself. In view of many indications in this and other groups of plants, this attitude needs to be much changed before we can lay a sound foundation for plant morphology. In records of cultures of the Saprolegniaceae it is still frequent to see flies, wasps, white of egg, this, that, and the other substratum mentioned without further details, as if the organism were assumed to act alike on all.

"*Saprolegnia monilifera* de Bary" has, so far as I know, been observed and studied only by de Bary himself. He found it only in one lake, near the Black Forest, Germany, from which he obtained it repeatedly, but was never able to get it from any other of his collections made through many years. De Bary considered its nearest relative to be *Saprolegnia torulosa* of the "*ferax*" group; but he placed it in a separate section which he called the "*monilifer*" group. Its anomalous position was thus clearly recognized by de Bary, and since this was the only case known to him in which both types of secondary sporangia were present, de Bary's native conservatism naturally induced him to attach it to one of the older genera.

An addition to the species which have this anomalous character of two modes in the formation of secondary sporangia during a sexual reproduction, was made recently by Coker (*l.c.*). He referred his species to the genus Achlya, as "*A. paradoxa*," and felt disinclined to erect for it a new genus, on the ground that the anomalous character in question establishes merely a "narrow point of contact" and is not unlike similar intermediate species in Puccinia and Uromyces. While this is clearly the case, limitations to genera cannot go on indefinitely encircling more and more exceptions, else one of the main reasons for the building up of the scientific classification of plants becomes abortive. Furthermore, in a group of such a small number of species as the Saprolegniaceae, the case becomes very different, by lack of balance, from the case in the genera of rusts cited. It seems to me, then, that by the erection of the genus Isoachlya, a double objective is attained: the limitations of the old genera remain clearly defined, and the new genus takes a most natural place within the family of the Saprolegniaceae.

Botanical Laboratory,
University of Michigan

LITERATURE

The student is referred to the extensive bibliography by Klebs for the older papers, and to Weston for more recent work.

Klebs, G. Zur Physiologie der Fortpflanzung einiger Pilze. II. *Saprolegnia mixta.* Jahrb. Wiss. Bot. 33: 513–593. 1899.

Weston, W. H. Observations on an Achlya lacking sexual reproduction. Amer. Jour. Bot. 4: 354–366. 1917.

EXPLANATION OF PLATES

The higher magnification was obtained with Bausch and Lomb 15 × ocular and 3 mm. objective, the lower with 15 × ocular and 8 mm. objective. All are equally reduced. Figures 1–9 are from cultures on sterilized house-flies, in 25–40 cc. conductivity water, from single zoospores; all cultures were free from other organisms.

Plate XIII

Fig. 1. Sporangial initials of *Isoachlya toruloides* on head of a fly, two days after inoculation.

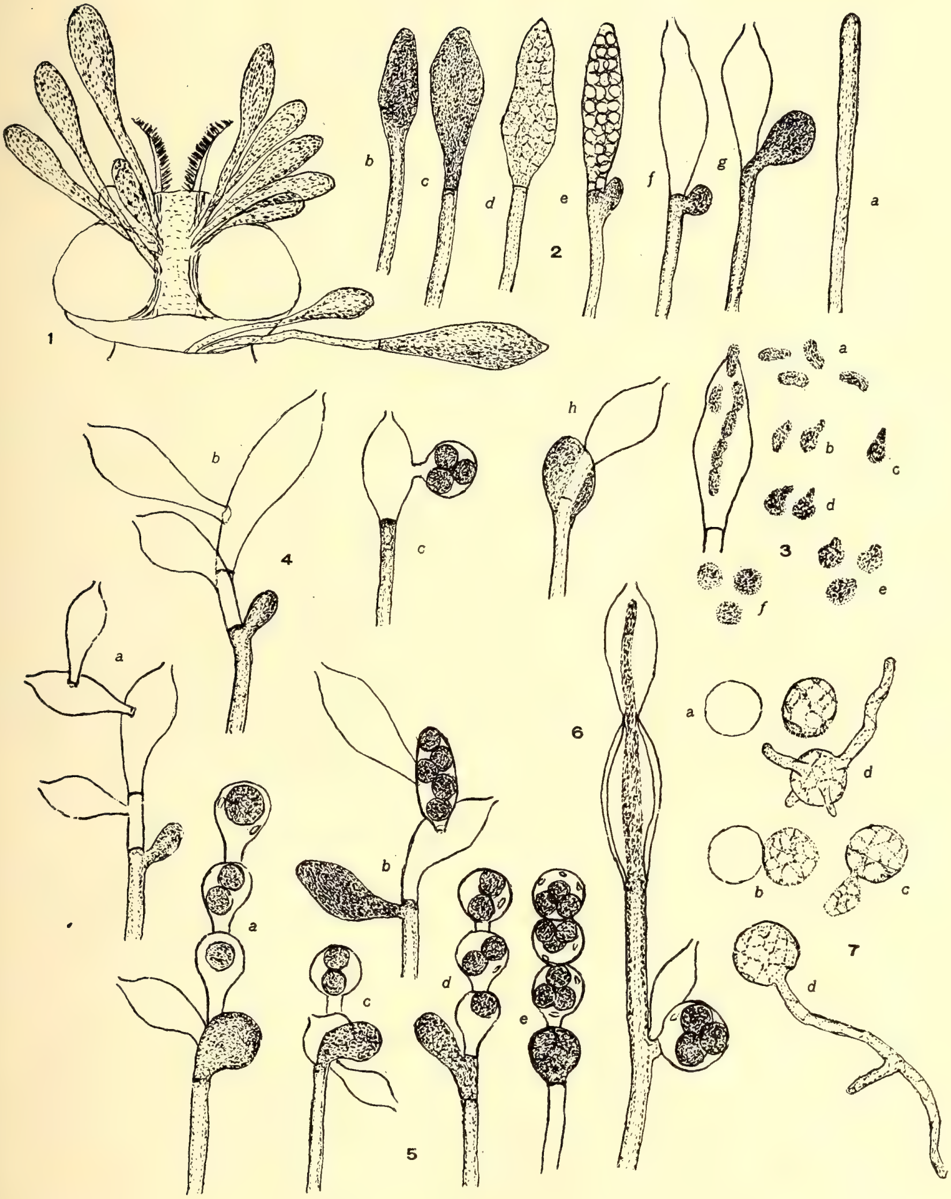
Fig. 2, *a*. Young hypha before swelling. *b–h*. Stages in the development and maturity of zoosporangia.

Fig. 3, *a–f*. Escaping zoospores and form changes in the process of assuming the first resting stage. *a*. During first twenty minutes. *b–e*. Gradual changes during next thirty minutes. *f*. Completely rounded and at rest. No cilia are shown, since full data were not obtained, but they are doubtless two in number and lateral.

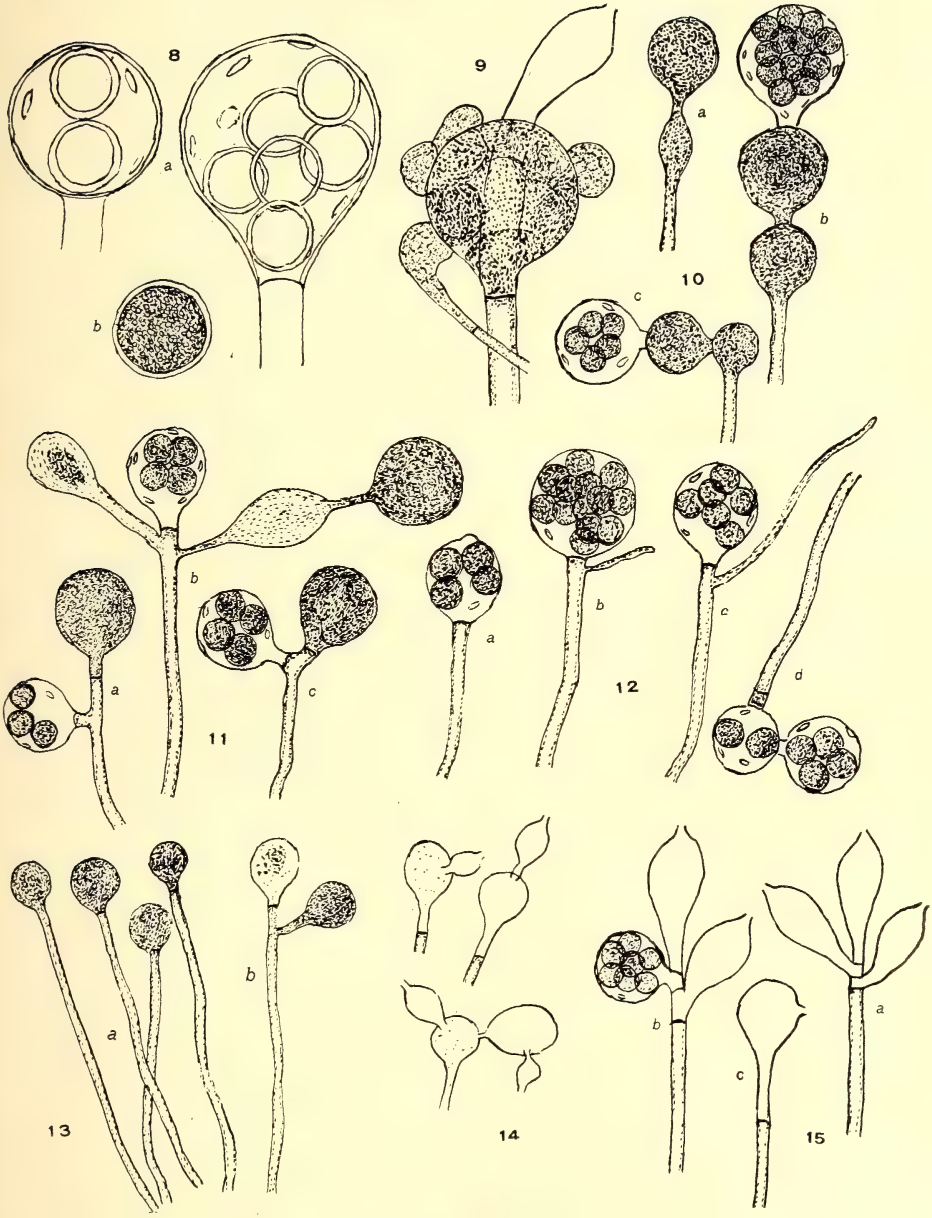
Fig. 4, *a–c*. "Achlya" type of formation of secondary sporangia, after nine days.

Fig. 5, *a–e*. Different types of oogonia, showing the normal variation in number of oospores as the fungus occurs on flies. After nine days.

Fig. 6. "Saprolegnia" type of formation of secondary sporangia. The proliferation in the two empty sporangia would become the third in the series. After thirteen days.


1
b
c
d
e
f
g
a
2
a
b
c
d
3
e
f
b
4
c
h
a
6
a
d
b
c
7
d
a
b
c
d
e
5

KAUFFMAN: ISOACHYLA.


8
a
b
9
a
b
10
c
b
11
a
c
12
a
b
c
d
13
a
b
14
15
b
c
a

KAUFFMAN: ISOACHYLA.

FIG. 7. After the end of the first swarming stage. *a.* Empty spore walls. *b.* Escaped but quiescent, naked cells. *c.* Act of escaping of cell. *d.* Germination by one or more germ tubes, the second "swarming" being omitted.

PLATE XIV

FIG. 8, *a.* Two examples of oogonia with mature oospores. *b.* A single mature oospore, much enlarged. After thirteen days.

FIG. 9. One of few occurrences of an oogonium with antheridia. Antheridia nearly mature; oogonium young. After 8 days.

FIG. 10, *a–c.* Oogonial arrangement in solution of haemoglobin 0.05% + KNO_3 0.1%. Note the larger number of oospores per oogonium. After four days.

FIG. 11, *a–c.* Oogonial arrangement in solution of haemoglobin 0.05% + levulose 0.12%. After twelve days. 90% of oogonia occur in pairs.

FIG. 12, *a–c.* Oogonia arranged singly at ends of long stalks, frequently with vegetative outgrowths below the oogonium, and oospores rather numerous. From culture in 0.1% leucin. *d.* In 0.02% leucin. In this weaker solution the inhibitive effect of leucin for the torulose arrangement is reduced. Both after ten days.

FIG. 13, *a.* In 0.02% peptone, after five days. *b.* After ten days. The oogonia are unable to mature in this solution.

FIG. 14. From peptone cultures in condition shown by figure 13, washed in distilled water for an hour, then transferred to distilled water. The initials become zoosporangia and the zoospores have escaped. After twenty-four hours.

FIG. 15. From culture in 0.02% peptone. *a.* Note regular arrangement of the basal walls of sporangia. *b.* Neat illustration of the doctrine of homology of different organs of reproduction. One of few oogonia which produced oospheres in this solution. *c.* An oogonial initial becoming a zoosporangium with scarcely any external morphological change.

THE TRANSMISSION OF RHUS POISON FROM PLANT TO PERSON[1]

JAMES B. McNAIR

(Received for publication January 3, 1921)

The progress of our knowledge of the transmission of Rhus poison from plant to person reflects, in a general way, the development of our understanding of plants and plant products. This is shown prominently in tracing the history of experiments in regard to the volatility and chemical nature of the poison. In this connection it may be well to consider, besides the dermatitant from *Rhus diversiloba* T. & G., the similar irritant substances from *R. Toxicodendron* L. and from its other sub-species *R. radicans* L.

The earliest explanation of Rhus poisoning attempted was that the plant gives off an invisible colorless vapor, or emanation, which, when breathed or permitted to touch the skin, causes dermatitis. The North American Indian and negro shared in this belief (Thompson, 44).

Some early writers associated Rhus poisoning with the fabulous stories told of the effects of the deadly upas tree (*Ipo toxicaria* Pers., *Antiaris toxicaris* Lesch.) of Java (Bennett, 4).[2]

The theory that the poison is non-volatile has also had its adherents.

---

[1] The substance of this paper was presented before the Graduate Botanical Club of the University of Pennsylvania, May 6, 1918.

[2] More light on the early physical and chemical nature of the principal irritant poison of this plant may be obtained through a study of the writings of Monti, Hunold, Gleditisch, Achard, Willemet, Pornai, and Krüger. All of these investigators considered the poison volatile. That this conclusion should be drawn at that time is not so remarkable if we consider that the gaseous exchange in plants was not understood at that time. Although Priestly (39) found in 1772 that plants give off oxygen, subsequent repetition of his experiment did not always give the same result. Ingenhousz (20) showed that the air was purified by plants in sunlight. He concluded, however, that the atmosphere is made injurious to animals by emanations from all plants in the shade and at night. Not only were all plants supposed to give off volatile poisons in the shade or at night, but Conradi, Ackerman, and Krauss believed the chief cause of various infectious diseases to be gaseous. As a result we have to this day the word malaria.

ACHARD, F. K. Nouv. Mem. de l'Acad. Royal de Sciences et Belles Lettres 1: 48. 1786.

ACHARD, F. K. Nachricht von Versuchen die über den Giftbaum (*Rhus Toxicodendron* Linn.) angestellt worden, um seine Bestandtheile zu kennen, und die Art und Weise, wie sein Gift auf verschiedene Thiere wirkt, zu bestimmen. Chemische Annalen von Lorenz Crell 1: 387–395, 494–503. 1787.

ACKERMANN, J. F. Von der Natur des ansteckenden Typhus; dem Wesen des Ansteckungsstoffs, der Art sich gegen denselben zu sichern, und der Methode die Krankheit zu heilen. Heidelberg, 1814.

CONRADI, —. Grundriss der Pathologie und Therapie 1: 288.

GLEDITISCH, J. G. Nouvelles expériences concernant les dangereux effets que les ex-

In 1788 Du Fresnoy, experimenting with *R. radicans*, steam-distilled its flowers and leaves. The distillate was not poisonous, but the residue in the still remained toxic.

Fontana (13) experimented with *R. Toxicodendron*. Because of his marked susceptibility to the poison he was forced to stop before he had determined whether or not the poison is volatile.

Two years later, Van Mons (45) collected about fifteen cubic inches of gas given off by a plant of *R. radicans*. Chemical experiments were carried on with this. He then engaged his brother, who was very sensitive to the poison, to hold his hand for more than one hour under a glass bell jar containing gas from the plant obtained in the middle of the day. A month later, not having noticed any eczematous symptoms, he repeated the same experiment with gas collected under a cylinder covered with black cardboard. He felt, even during the immersion, a burning sensation, and developed a typical case of Rhus dermatitis. Van Mons concluded that the poisonous principle of *R. radicans* is a gaseous hydrocarbon which emanates from the plant only at night, on cloudy days, or in the shade.

In 1798, Horsfield, a medical student at the University of Pennsylvania, stated that some people were affected by the exhalations of *R. Vernix* and *R. radicans* to a distance of twenty feet from the plant. He also noticed that dermatitis was produced by the immediate application of the juice of the plant to the external surface of the skin. In analyzing *R. radicans* he placed two pounds of the flowers and leaves with several quarts of water in a small copper still. The distillate was not poisonous, but the residue in the still retained its toxicity.

Lavini (25) considered the poison of *R. Toxicodendron* a gum resin, mixed with a "subtil" acid principle, qualified to combine with the hydrocarbon gas which emanates from the plant after sunset. According to him, the effect of the sap squeezed from the leaves is analogous, but less intense. The effect of "water" distilled from this plant was still less intense.

Khittel (22) attempted a more thorough chemical analysis of *R. Toxicodendron*. Because of his inability to find the poison by the processes outlined, he considered it a volatile alkaloid.

halaisons d'une plante de l'Amerique Septentrionale produisent sur le corps humain. Mem. de l'Acad. de Berlin 61–80. 1777. Ditto. Journal de Physique **21**: 161–175.

Hunold, —. Piepenbrugs Archiv für die Pharmac. 1: 279.

Kraus, J. C. Verhandeling over der aard en de werking det geneeswiddelen, welke ter bestrijding van zenuwkwalen en derzelver toevallen worden Aangewend. Amsterdam, 1819.

Krüger, —. Archiv für die Pharmac. 1: 261.

Monti, Guiseppe. De plantis venenatis. Accademia della scienze dell' Instituto di Bologna. Commentari II, **3**: 160–168. 1755.

Pornai, —. Giornale per sevire alla storia ragionata della medicinia di questo secola. Venezia 1: 83. 1783.

Willemet, Remi. Observations sur les effets du *Rhus radicans*. Jour. de Phys. **51**: 369–370. 1800. Ditto. Journ. de Med., Chir., Pharm., etc., Paris 1: 209–211. 1801.

Millon (35), evidently unaware of the work of Khittel, also investigated *R. Toxicodendron.* He believed the poison a non-volatile gum resin requiring direct contact to cause dermatitis. He found its alcoholic solution to be toxic.

Discussing the experiments of Khittel, Maisch (33) held the poison to be volatile and said:

> It is natural to suppose that, during the process of drying, the greatest portion of the poisonous principle should be lost. This must be still greater, if the dried leaves are powdered, a hot infusion prepared from them, and this infusion evaporated down to the original weight of the dried leaves. It is obvious that Khittel could not have selected a better method for obtaining the least possible quantity of the poisonous principle, if, indeed, it could be obtained by this process at all.

Later Maisch (34) disagreed with Khittel and denied the presence of a volatile alkaloid. He thought that he had found a new volatile acid, which he held to be the active principle and which he called "toxicodendric acid." Maisch enclosed in a tin box a lot of freshly collected leaves of poison ivy, and introduced into this box a number of moistened test papers. The next morning he found that the blue litmus paper had been colored strongly red, whereas curcuma and red litmus paper were unaffected. He writes regarding this experiment:

> This single experiment was at once a conclusive proof that the exhalations of these leaves contained a volatile acid, and that the poisonous properties were most likely due to it.

Maisch describes further how he obtained an impure watery solution of his toxicodendric acid by maceration of the leaves, expression and distillation of the expressed liquid. In preparing his acid, he suffered from a copious eruption and the formation of numerous vesicles on the back of his hands, fingers, wrists, and bare arms. He says further:

> Several persons coming into the room while I was engaged with it were more or less poisoned by the vapours diffused in the room, and I even transferred the poisonous effects to some other persons merely by shaking hands with them. The dilute acid, as obtained by me, and stronger solutions of its salts, were applied to several persons, and eruptions were produced in several instances, probably by the former, though not always, which was not likely owing to the dilute state of the acid.

Maisch did not isolate his acid nor any one of its salts; he never had the substance in question chemically pure. He proved only the presence of a volatile acid. He noticed the characteristic eruption on his own skin while working with the poison ivy. Persons coming to the laboratory at this time were often poisoned. He observed also that an eruption sometimes followed the application of the impure solution of this acid to the skin. From these very rudimentary experiments he drew the wholly unwarranted conclusion that his acid must be the active principle.

By far the most valuable work on *Rhus Toxicodendron* is that of Pfaff (37). From a clinical study of Rhus poisoning, Pfaff came to the conclusion that the poison must be a non-volatile skin irritant. The more volatile the irritant, the quicker is its action on the skin. Formic acid acts very quickly; acetic acid, less volatile than formic, acts more slowly, but still much more quickly than poison ivy, the latent period of which is usually from two to five days. Pfaff thought that the volatile acid obtained by Maisch might have contained some of the poisonous principle as an impurity, but that it could not produce the dermatitis if prepared in a pure state. He therefore prepared a quantity of the acid by distilling the finely divided fresh plant with steam. The yield was increased by acidulating the mixture with sulphuric acid before the distillation. The acid distillate so obtained was freed from a non-poisonous oily substance by shaking the solution with ether. Barium and sodium salts were made by neutralizing the acid and were purified by crystallization. Analysis showed them to be salts of acetic acid, and they gave the characteristic tests for this acid. The "toxicodendric acid" of Maisch was thus shown to be acetic acid, and not therefore the poisonous principle of the plant.

Pfaff obtained the active principle by the process which he outlines. The lead compounds made in different preparations were analyzed and assigned the formula $C_{21}H_{30}O_4Pb$. The oil itself was not analyzed. Pfaff proposed the name toxicodendrol for the oil. He found that it is not volatile, is decomposed by heat, is soluble in alcohol, ether, chloroform, benzene, etc., but insoluble in water. Its effects upon the human skin were studied in many experiments upon himself and others. It was shown that an exceedingly minute quantity of the poison will produce the dermatitis, even 1/1000 milligram applied in olive oil being active. The oil was given internally to rabbits, its effects being most marked on the kidneys.

Acree and Syme (1) found gallic acid, fisetin, rhamnose, and a "poisonous tar, gum, or wax" in the extract prepared by maceration of the leaves and flowers of poison ivy with ether, and subsequent distillation of the solvent. The lead compound of this poisonous substance was found to be soluble in ether. The authors utilized this property to free the poisonous material from admixed non-poisonous substances. Lead compounds were first prepared by precipitating an alcoholic solution (of the ether extract of the drug) with lead acetate. The precipitate was washed with water, partially dried over sulphuric acid, placed in a Soxhlet apparatus, and extracted with ether until the solvent came over colorless. A green solution was obtained which was washed with water and decomposed with hydrogen sulphide. On evaporating the solvent, a black, poisonous "tar or gum" remained. Upon hydrolysis with 2 percent sulphuric acid, this poisonous substance gave fisetin, rhamnose, and gallic acid. The residue in the thimble was decomposed by hydrogen sulphide, shaken with ether, and evaporated. A hard, brittle, yellow, non-poisonous resin was obtained. The authors believe the poisonous principle of poison ivy to be a complex substance of glucosidal nature.

Chyser in 1910 considered the poison of Rhus a toxalbumin formed by the combination of a liquid acid with albumin. He puts forth the following evidence in support of this conclusion: (1) the small amount of poison (0.000005 g.) necessary to produce itching and burning on the skin; (2) similarly to a toxalbumin, it loses its toxicity by heating to 50° C. on a water bath; likewise at 75° C. and 100° C. The toxicity was tested by rubbing with a probe on the skin of the upper arm. In no case was irritation evident. This evidence is inconclusive of the poison's being a toxalbumin, for: (1) other substances besides toxalbumins are poisonous when in such small amounts; (2) the poison remains toxic if heated on glass in a steam autoclave for one hour under twenty pounds' pressure per square inch (temperature 126.2° C.); and (3) the poison contains no nitrogen.

The work of Acree and Syme is probably erroneous for: (1) all three of the so-called constituents of the poison are found in two non-poisonous species of Rhus; (2) the natural glucoside yielding fisetin, rhamnose, and gallic acid is non-toxic; and (3) there is not sufficient evidence that the poisonous substance which Syme attempted to decompose was not a complex containing a poisonous body and one or more non-toxic glucosides in addition. McNair (30), working with *R. diversiloba*, concluded that the poison of this plant is not a glucoside of rhamnose, fisetin, and gallic acid. A different method was used for extracting the poison, and none of these substances could be obtained on hydrolysis.

The specific cause of skin poisoning from *R. Toxicodendron* L. and its two sub-species, *R. diversiloba* T. & G. and *R. radicans* L., has thus far been ascribed successively to: an emanation of vapor; a hydrocarbon gas; a gum resin, mixed with a "subtil" acid principle, qualified to combine with hydrocarbon gas which emanates from the plant after sunset; a volatile alkaloid; a non-volatile gum resin; a volatile organic acid (toxicodendric acid); an infection by bacteria (*M. toxicatus*, Burrill, 7); a non-volatile oil (toxicodendrol); a glucoside of fisetin, rhamnose, and gallic acid (toxicodendrin); a toxalbumin; and finally to something other than a glucoside of fisetin, rhamnose, and gallic acid.

The Transmission of Rhus diversiloba Poison

My investigation of the transmission of the poison has been carried on from three standpoints; botanical, chemical, and pathological. The following chemical experiments were carried out:

1. One half pound of fresh, finely chopped poison oak leaves were distilled normally at different temperatures up to the point of decomposition of the leaves. As a result, both the distillate and the residue were non-toxic.

2. Another lot of leaves similarly prepared was subjected to steam distillation. The distillate was non-toxic, but the residue in the retort remained toxic.

3. Distillation, either destructive or with ether, when done under reduced pressure, gave non-toxic distillates.

From the results of these distillation experiments it can be safely argued that the poison is non-volatile and that if non-volatile it can not be carried by entrainment with a volatile substance. It has been considered by some as a non-volatile poison carried by a volatile oil.

In the investigation of the smoke of the burning plant (Von Adelung, 46), leaves were placed in a glass combustion tube. The glass tube was then heated until the leaves began to smoke. The smoke was blown against the skin of a susceptible individual. Dermatitis resulted. The experiment was repeated with the addition of cotton plugs in each end of the tube. Dermatitis did not result.

It was thought that perhaps condensation of the irritant might have occurred on the cotton. The experiment was therefore repeated (McNair, 30), glass wool plugs being used instead of cotton. The glass wool was kept at the same temperature as the burning leaves. No dermatitis resulted. It is concluded, therefore, that the non-volatile poison is carried by particles of soot in smoke.

It is also possible to determine the non-volatility of the poison physiologically. A fresh leaf of poison oak was lightly glued to the concave side of a watch glass about six inches in diameter. The watch glass was then taped on the breast of a susceptible person (the concave side inward) and left there for half an hour. No dermitis resulted. The foregoing experiment was repeated, substituting for the leaf a drop of sap. No ill effects resulted.

A drop of sap was now placed on the skin of a susceptible individual, and the area was covered by a watch glass. Dermatitis occurred after a few hours, but only in the area to which the sap was applied. It did not spread. If the poison were volatilized with moderate ease, at ordinary temperatures, it would have caused a general irritation at, as well as around, the area to which it had been applied. Volatile poisons rapidly penetrate into the tissues, and diffuse there with great ease. Such is the case with the various oils of turpentine, many ethereal oils from the vegetable kingdom, and numerous substances belonging to the aliphatic series, *e.g.*, chloroform and ethyl chloride. Petroleum, benzol, and other compounds of the aromatic series cause local irritation in essentially the same way (Schmiedeberg, 41).

In another experiment, sap was placed on the skin of a susceptible person. After dermatitis had occurred, the affected section of the skin was cut out and thin sections were mounted on microscopic slides. These sections showed that the poison had penetrated but slowly in the skin (McNair). If the poison were volatile, penetration would occur more rapidly and diffusion would be greater.

In ordinary cases of Rhus poisoning, dermatitis is not noticed until about twelve hours or more after exposure. This long period of latency is much against the supposition that the poison is volatile. It would be much easier for a volatile poison to evaporate and diffuse through the atmosphere in twelve hours if it required a dozen hours to penetrate the skin.

From the preceding experiments, it is clear that the poison is non-volatile. But we still have the question to answer as to how poisoning occurs without contact with the plant. This question has been studied by Von Adelung (46), Schwalbe (43), Hubbard (17), Hadden (16), and Frost (15).

Von Adelung considered the pollen to be toxic and disseminated by the wind. As a matter of fact, the pollen may be rubbed on the skin of a susceptible person without ill effects. The skin may even be lacerated. The pollen grains, although small enough to be carried by the wind, have no wing-like projections or tissues which would aid their flight, but on the contrary are covered with a sticky substance which tends to hold them in masses to the flower. Pollination is effected by insects. Similar non-toxic results have been obtained with the pollen of other poisonous species of Rhus; with that of *R. vernicifera* by Inui (21), that of *R. Vernix* by Warren (48), and that of *R. Toxicodendron* by Rost and Gilg (40).

Schwalbe (43) attributed poison transmission to the trichomes of the plant. The trichomes are very minute and are found in abundance on the young stems and on the under surfaces of the leaves. The trichomes were considered to be poisonous and carried by the wind.

In an investigation of this theory, fresh leaves were placed in an alembic, and a current of air was blown through. The outcoming air current was caused to impinge on the skin of a susceptible individual. No dermatitis resulted. The experiment was repeated, except that the outcoming air was caused to bubble for several hours through alcohol in which the poison is soluble. This alcoholic solution was concentrated and found to be non-toxic. In another experiment, the hairy side of an uninjured leaf (previously examined carefully with a hand lens for the absence of droplets of sap) was drawn across the skin with no ill effects. In another test an uninjured leaf was placed in 95 percent alcohol at room temperature for ten minutes. The alcoholic solution was concentrated and found to be non-toxic.

Rost and Gilg (40) carried on experiments with *R. Toxicodendron* to determine if the plant hairs drop off spontaneously, if they can be blown off from cut twigs, and if the poison, as in *Primula obconica*, can be obtained by contact from the under sides of the leaves. Two shells containing glycerine were placed under Rhus plants for two windy days in May. When this liquid was examined microscopically after the experiment, needle-shaped and club-shaped hairs were found. On October 17, 1911, three wide glass dishes containing glycerine water were placed under thickly leaved branches of *R. Toxicodendron*. These were left for four days. A microscopical examination on October 21 showed no hairs in the dishes.

The preparations contained considerable dust. From the results of these experiments, it is evident that the hairs do not drop off to any great extent spontaneously at either the beginning or the end of the vegetative period.

To determine whether or not the trichomes could be forcibly blown off, five experiments were conducted in 1911:

A. At the end of July (Exp. S. 1 and 2);
B. At the end of August (Exp. S. 3);
C. At the end of September (Exp. S. 4);
D. After the middle of October (Exp. S. 5).

A branch was firmly fastened within a rectangular glass case (100 × 75 × 180 cm.) and was exposed to an air current of about 0.3 atmosphere pressure from a distance of approximately 15 cm. so that the leaves moved as if in a storm. The air current, after passing the leaves, struck an inclined glass plate on which were placed glycerine-covered slides. The current then left the case through a funnel closed with cotton. On the bottom of the glass case two more glycerine-covered slides were placed. During experiments the air current was often interrupted, especially at the beginning and towards the end. This was done to secure the strongest possible disturbances of the leaves. Each experiment lasted at least two hours. Freshly cut branches were used. These branches were afterward pressed and stored, for microscopical examination as to the presence of trichomes. Trichomes were found to have been left on the leaves in abundance.

The glycerine-moistened slides were examined under high and low magnifications. At the end of each experiment, preparations of the dried leaves were made in a chloral-hydrate solution to find if hairs still remained.

The branches used were:

In Series 1:		In Series 2:	
1st day	fresh	1st day	fresh
2d day	one day old	2d day	one day old
3d day	two days old	3d day	three days old

A. *Experimental Series 1* (July 26–28, 1911). Herbarium specimens and two microscopical cross sections gave evidence of many hairs.

I. Wednesday, July 26. The experiment lasted 11 hours. During the first hour the position of the branch was changed twice. A microscopical examination of the glycerine-moistened slides on July 27 showed the absence of club-shaped hairs, but the presence of needle-shaped hairs, much dust, and other impurities.

II. Thursday, July 27. The branch used in the foregoing experiment was exposed to the blast again for two hours (from 11:15 A.M. to 1:15 P.M.). When examined microscopically on July 28, the preparations showed that the dried-up branch as well as the fresh one had not given off club-shaped hairs but only needle-shaped hairs.

III. Friday, July 28. The almost entirely dried branch was subjected for the third time to the air blast (from 10:00 A.M. to 12:00 M.). A microscopical examination on the same day (July 28) showed the presence of needle hairs in all preparations, but of only one club-shaped hair.

At the end of experimental series 1, a chloral-hydrate preparation was made of the entirely dried branch. The under side of the leaves, as is the case in the fresh leaf, were covered with many club-shaped and bristle-like hairs.

B. Experimental Series 2 (July 28–31, 1911). In this series of experiments a branch of densely haired *R. Toxicodendron* was used. A part was pressed and a chloral-hydrate preparation made of it. This showed a dense covering of both kinds of hairs.

I. Friday, July 28. The experiment lasted from 12:30 to 2:30 P.M. On July 29, a microscopical examination disclosed a club-shaped hair in each of four preparations; the remainder showed many needle-shaped hairs.

II. Saturday, July 29. The dried branch was blown on for two hours (from 11:00 A.M. to 1:00 P.M.). A microscopical examination followed on Monday, July 30. This disclosed in

Preparation 1: Three club-shaped hairs, very many needle-shaped hairs, many dirt particles, and pollen grains of other plants.
Preparation 2: No club-shaped hairs.
Preparation 3: Four club-shaped hairs, one with a piece of epidermis.
Preparation 4: Two club-shaped hairs.
Preparation 5: Two club-shaped hairs, one containing yellow protoplasm.
Preparation 6: One club-shaped hair.
Preparation 7: Three club-shaped hairs.
Preparation 8: No club-shaped hairs.

III. Monday, July 31. The three-day-old branch was blown on from 11:30 A.M. to 1:30 P.M. A microscopical examination on August 1 showed one club-shaped hair in each of four out of eight preparations. Both the glycerine-covered slides on the bottom of the case were free from club-shaped hairs. The cotton in the funnel contained no club-shaped hairs (the cotton having been soaked in glycerine and the excess pressed out).

At the end of the experiment, a chloral-hydrate preparation was made from the three-day-old, entirely dried branch. Club-shaped hairs were present in abundance on the leaves. The club-shaped hairs could never wound the cuticle.

Three further experiments were made, similarly to the first two, toward the end of August, in the second half of September, and soon after the middle of October, 1911. The results were similar. In the first days none, or at most one or two, club-shaped hairs could be found in 8 to 10 preparations. In the experiments with the twigs dried two or three days, only a few club-shaped hairs were blown loose. In many experiments in which preparations of hairs were spread on the skin, not the slightest irritation appeared.

The glycerine layer of one or more experiments was applied and dried on the uninjured skin of the under side of Rost's forearm. The results were negative. Rost was susceptible to the resinous sap of the same shrub. It seems evident, therefore, that the trichomes are non-toxic and are not a means of conveyance of the poison from plant to person.

Hubbard (17) and Hadden (16) thought insects might carry Rhus poison from the plant in ways similar to those by which flies carry bacteria from place to place. This method of transmission seems hardly practicable in many cases. It should be borne in mind that the insect could not transmit the poison by coming in contact with the uninjured plant.

Recently, Frost (15) believed the poison to be bacterial. This has been refuted (McNair, 32).

The methods already discussed constitute all that have been suggested for the transmission of Rhus poison to a distance. As none of them prove very serviceable, we still have to consider the question as to how poisoning occurs without contact with the plant.

It has been found in an examination of the sap that: (1) The unelaborated sap of the xylem is non-toxic; (2) the elaborated sap of the phloem is non-toxic; and (3) the resinous sap of the resin canals is poisonous.

A further examination of the plant tissues shows that the xylem, epidermis, and trichomes which do not contain the resin canals are non-toxic.

When the flowers are examined, it is evident that resin canals do not extend more than half-way up the fully matured stamens, and so it would be expected that the pollen would be non-toxic. The flower of the female plant, on the other hand, contains resin canals in the pistil, and an abundance of resin canals surround the ovule. The ovule remains highly toxic until the seed has fully ripened. The poison, therefore, acts as a protection to the immature seed. This plant thus exemplifies the natural law developed by Kipling (23) that the female is more deadly than the male. It has also been shown (McNair) that the maximum number of cases of Rhus dermatitis recorded in the University of California Infirmary occurs previous to the opening of the flowers.

It has long been known that fresh leaves are more likely to produce poisoning than are dry or fallen leaves. This difference in malignancy has been attributed to a poisonous gas given off by the plant.

Van Mons (45) was convinced by the large number of cases among persons of his acquiantance, that the evil effects of Rhus were produced by a gaseous substance which escaped from the living plant, because the dry leaves or fallen leaves never caused trouble.

Professor Asa Gray also held this same opinion in 1872, as the following letter to Dr. J. C. White discloses:

> My personal knowledge that Rhus dried specimens are harmless amounts merely to

> this: I handle over and over dried specimens with impunity, but am very sensitive to the fresh plant. Then the poison is volatile, as shown by its affecting persons who do not touch it actually; that of the leaves, I should say, must escape and dry out in the drying process, or in the course of time. In a stem it would not volatilize so soon; but I should not expect to be poisoned from any *old* herbarium specimen, either from twigs or leaves.

Likewise, Mackie (28), writing on the value of oak leaves for forage, says:

> It would seem that the irritating and poisonous oil of poison oak is volatile at a comparatively low temperature. In gathering the specimens the writer was badly poisoned even though gloves were worn; yet after drying at ordinary room temperature, and the leaves pressed into the mill with bare hands, no poisoning effects followed.

Opposed to these opinions is the experience of Bogue (6) while investigating an herbarium specimen of *R. venenata* which had been deposited in the Ohio State University not less than three years. He was poisoned by the "sawdust" from the stems of the plant which was the result of borings from a beetle.

It has previously been conclusively shown that the poison is non-volatile, and the decrease in malignancy of the leaves in drying can be attributed only to a loss of fluidity of the sap and to the loss of toxicity of the poison from oxidation (McNair).

In concluding the botanical investigation, it seems evident that the plant is capable of poisoning only when injured in such a manner that the poisonous resinous sap exudes.

Poisoning without contact with the plant may occur by means of smoke from the burning plant or by contact with substances that have the poisonous sap on them, such as gloves (Hunt, 18; Ward, 47; Frost, 14; Kunze, 24); pocket-knife handles, croquet balls, and botanists' collecting cases (Hunt, 18); hands of another (Hunt, 18; White, 49; Planchon, 38; Cantrell, 10; Maisch, 34); clothing (Balch, 2; White, 49; Bibb, 5; Lindley, 27; Cundell-Juler, 11); shoes one year after contact (Balch, 2; Ward, 47); instruments (Planchon, 38); leather hat bands (Leonard, 26); and firewood (Barnes, 3).

Dermatitis caused by other plants is also sometimes attributed to Rhus; *e.g.*, Cypripedium (Hurlbut, 19); eczema and other eruptions may also be confused with that caused by Rhus.

Conclusions

1. The principal dermatitant of *Rhus diversiloba* is not volatile, for:

(*a*) It is not distillable normally by steam or under reduced pressure. It can not be carried by entrainment with a volatile substance.

(*b*) The smoke of the burning plant is not poisonous when filtered through glass wool at a high temperature.

(*c*) Possible emanations from leaves are non-toxic when (1) the leaves are fastened on the concave side of a watch-glass and then to the skin of a susceptible person; and (2) when a current of air is blown over the leaves and caused to bubble through alcohol, the alcohol is non-toxic.

(*d*) Dermatitis occurs only on the area of skin to which the poisonous sap has been applied; a general irritation as by volatile irritants is not produced.

(*e*) It does not diffuse rapidly in the skin, as is shown microscopically in sections of diseased skin.

(*f*) The period of latency is too long.

2. Portions of the plant which do not cause dermatitis are: the pollen, the trichomes, the epidermis, the cork cells, and the xylem.

3. The poison is confined exclusively to the resinous sap.

4. Leaves decrease in malignancy in drying from the loss of fluidity of the sap and from the oxidation of the poison.

5. Poisoning without contact with the plant may occur from the smoke of the burning plant or by contact with substances that have the poisonous sap on them, such as clothing, shoes, cordwood, tools, the hair of animals, etc.

6. Dermatitis caused by other plants is sometimes attributed to Rhus. There is difficulty in distinguishing eczema from Rhus dermatitis.

LITERATURE CITED

1. **Acree, S. F., and Syme, W. A.** On the composition of toxicodendrol. Jour. Biol. Chem. **2**: 547. 1906–1907.
2. **Balch, A. W.** Poison ivy. Jour. Amer. Med. Assn. **46**: 819. 1906.
3. **Barnes, E.** The poison ivy. Med. Rec. **30**: 157–158. 1886.
4. **Bennett, J. J.** Plantae javanicae rariores descriptae iconibusque illustratae. Pt. 1, p. 60. London, 1838.
5. **Bibb, L. B.** Experimental Rhus poisoning. Texas Med. Jour. **30**: 162–163. 1914–1915.
6. **Bogue, E. E.** Garden and Forest. 1894.
7. **Burrill, T. J.** Some vegetable poisons. Amer. Monthly Micr. Jour. **3**: 192–196. 1882.
8. ——. Some vegetable poisons (Abstr.). Proc. Amer. Assn. Adv. Sci. **3**: 515–518. 1882.
9. ——. Rhus poisoning. Garden and Forest **8**: 368–369. 1895.
10. **Cantrell, J. A.** Unusual mode of transmission in a case of dermatitis venenata. Med. News **59**: 484. 1891.
11. **Cundell-Juler.** The poison vine. Cincinnati Lancet and Clinic n.s. **11**: 73–76. 1883.
12. **Du Fresnoy, de V.** Des caractères, du traitement et de la cure des dartres et de la paralysie, etc., par l'usage du *Rhus radicans*, Vol. 1. Paris, an VII.
13. **Fontana, F.** Trattato de veleno della vipera de velem American **1**: 148; **3**: 114–117. Naples, 1787. Eng. transl. by Joseph Skinner. 2nd ed. **2**: 181–184. London, 1795.
14. **Frost, J.** Remarks on the erysipelatous inflammation produced by the juice of *Rhus Toxicodendron*. London Med. Physical Jour. **55**: 116. 1826.
15. **Frost, L. C.** The bacterial etiology of poison oak dermatitis (Rhus poisoning). Med. Rec. **90**: 1121–1123. 1916.
16. **Hadden, A.** Poison ivy or *Rhus Toxicodendron*. Med. Review of Reviews **12**: 764. 1906.
17. **Hubbard, S.** Rhus poisoning. Med. Brief **32**: 884. 1904.
18. **Hunt, J. H.** Rhus poisoning. Brooklyn Med. Jour. **11**: 392–406. 1897.
19. **Hurlbut, E. T. M.** Antidote to poison oak. Calif. Homeopath **7**: 235–239. 1869.
20. **Ingenhousz, J.** Experiments upon vegetables, discovering their great power of purifying common air in the sunshine, and of injuring it in the shade and at night. London, 1779.
21. **Inui, T.** Ueber den Gummiharz-Gang des Lackbaumes und seiner verwandten Arten (Abstr.). Bot. Centralbl. **83**: 352. 1900.

22. **Khittel, J.** Chemische Untersuchung der Blätter des Giftsumachs (*Rhus Toxicodendron*). Witt. Viertelj. Prakt. Pharm. **7**: 348–359. 1858.
23. **Kipling, R.** The female of the species. A study in natural history. Ladies Home Jour. **28**: 11. 1911.
24. **Kunze, R. E.** Poison Rhus. Med. Tribune **5**: 111–120. 1883.
25. **Lavini, M.** Émanations délétères qui émanent *Rhus Toxicodendron.* Jour. Chim. Méd. **1**: 249–251. Paris, 1825.
26. **Leonard, W. W.** Rhus poisoning. Med. Chron. **3**: 21. 1884–1885.
27. **Lindley, J. S.** Rhus poisoning. Amer. Jour. Dermat. and Genito-Urin. Dis. **12**: 342–344. 1908.
28. **Mackie, W. W.** Value of oak leaves for forage. Calif. Agr. Exp. Sta. Bull. 150. 1903.
29. **McMaster, J. B.** Life and times of Stephen Girard. 1918.
30. **McNair, J. B.** The transmission of Rhus poison from plant to person. *Rhus diversiloba* T. & G. Jour. Infect. Dis. **19**: 429–432. 1916.
31. ——. Pathology of dermatitis venenata from *Rhus diversiloba.* Jour. Infect. Dis. **19**: 419–428. 1916.
32. ——. The poisonous principle of poison oak, non-bacterial. Med. Record **91**: 1042–1043. 1917.
33. **Maisch, J. M.** Proc. Amer. Pharm. Assn. **13**: 166–174. 1865.
34. ——. On the active principle of *Rhus Toxicodendron.* Amer. Jour. Pharm. **38**: 4–12. 1866.
35. **Millon, de Revel.** Recherches sur le gui de chêne, et nouvelles observations sur le sumac vénéneux, ou *Rhus Toxicodendron.* Bull. Acad. Méd. Paris **26**: 501–505. 1860–1861.
36. ——. Accidents-constatation d'immunité rapportée a la végétation. Jour. Méd. Chirug. et Pharm. Toulouse. Nov., 1862.
37. **Pfaff, F.** On the active principle of *Rhus Toxicodendron.* Jour. Exp. Med. **2**: 181–196. 1897.
38. **Planchon, L.** Accidents causés par le contact du *Rhus Toxicodendron* (Terebinthacées Anacardiées). Montpel. Méd. II., **9**: 61, 219. 1887.
39. **Priestly, J.** Experiments and observations on different kinds of airs. p. 324. London, 1774.
40. **Rost, E., and Gilg, E.** Der Giftsumach, *Rhus Toxicodendron* L., und seine Giftwirkungen. Ber. Deutsch. Pharm. Ges. **22**: 296–358. 1912.
41. **Schmiedeberg, O.** Grundriss der Arzneimittellehre. 3te Aufl. pp. 213–224. 1895.
42. **Schwalbe, C.** On the active principle of *Rhus diversiloba* (poison oak). Med. Record **63**: 855. 1903.
43. **Schwalbe, K.** Die giftigen Arten der Familie Rhus: *R. diversiloba, R. Toxicodendron* und *R. venenata.* Münchener Medic. Wochenschr. **49**: 1616. 1902.
44. **Thompson, W.** Challenger Expedition. Jour. and Proc. Ham. Assoc. **8**: 126. 1892.
45. **Van Mons, J. B.** Mémoire sur le *Rhus radicans.* Actes Soc. Méd. Chirurg. et Pharm. **12**: 136–137. 1797.
46. **Von Adelung, E.** An experimental study of poison oak. Thesis Univ. Calif. Nov., 1912. *Also* Arch. Intern. Med. **11**: 184. 1912.
47. **Ward, R. F.** Severe ivy poisoning. N. Y. Med. Jour. **88**: 1224. 1908.
48. **Warren, L. E.** Some observations on the pollen of poison sumach. Amer. Jour. Pharm. **85**: 545–549. 1913.
49. **White, J. C.** On the action of *Rhus venenata* and *Rhus Toxicodendron* on the human skin. New York Med. Jour. **17**: 225–249. 1873.

THE TYPE CONCEPT IN SYSTEMATIC BOTANY[1]

A. S. Hitchcock

(Received for publication January 8, 1921)

The binomial system of nomenclature has been an important factor in the development of taxonomy. The increase in the number of known species since the time of Linnaeus has been many fold; because of carelessness and ignorance the number of names applied to the species of plants has been much greater than the number of species; the increase in our knowledge of genetic relationships and the diversity of opinions among botanists concerning generic limitations have still further increased the synonymy. The confusion arising from these causes soon emphasized the need of a code of nomenclature by which the naming of plants might be regulated. Many codes have been proposed, but only two have received the support of international conferences: the Paris Code of 1867, and the Vienna Code of 1905.

I have pointed out in another place (Science n. ser. **30**: 597. 1909) that absolute stability in nomenclature is unattainable so long as botany is a growing science. The limits of genera will vary according to the knowledge and the opinions of individual workers, and the names of the plants as they are assigned to this or that genus will change in a corresponding degree. A universal code cannot bring about a permanent nomenclature, but it enables botanists to apply names according to definite rules, and this is all that we may expect of any code.

The two codes mentioned have been a great help in stabilizing nomenclature. Experience has shown, however, that they lack definiteness in directing the application of names, especially of generic names. In the early days of taxonomy a name was applied to a concept rather than to an entity. A generic name was based upon all the known species of the genus; a specific name was based upon all the known specimens of the species. When a genus was divided the original name was retained for one of the parts, usually the larger part, or was sometimes discarded altogether. The Vienna Code introduced many reforms, but the procedure for applying names when a genus or species was divided was still vague and uncertain in its application.

About 30 years ago a new system began to receive serious attention among American botanists, the system of applying names by means of types. It is not my purpose here to give a history of this idea, but rather to point out some of the advantages of the system. The type concept lies at the basis of modern botanical nomenclature. The type species of a genus or the type specimen of a species is the species or the specimen respectively that directs or controls the application of the generic or specific name. A generic name shall always be so applied as to include its type species; a specific name shall always be so applied as to include its type specimen. The old concept was that a genus was a group of species having a given combination of characters; a species, similarly, a group of specimens. The new or type concept is that, from the nomenclatural standpoint, a genus is a group of species allied to the type species, a species a group of individuals similar to the type specimen.

---

[1] Read before the Systematic Section of the Botanical Society of America at Chicago, December 29, 1920.

If a genus or species is divided, that part which includes the type species or specimen retains the generic or specific name, be this part relatively large or small. The American Code[2] recognized the type concept as a fundamental principle. The Paris and Vienna codes do not refer to this principle. But the idea had made such headway by 1910 that it was recognized by the Brussels Congress in a recommendation as a guide for the future (an addition to Recommendation XVIII). This reads:

> [Botanists will do well, in publishing, to conform to the following recommendations: XVIII . . .] XVIII *bis*. When one publishes the name of a new group, to indicate carefully the subdivision which is considered to be the nomenclatural type of the group; the type genus of a family, the type species of a genus, the type variety or the type specimen of a species. This precaution avoids the nomenclatural difficulties in the case where, in the future, the group in question comes to be divided. (Act. Congr. Internat. Bot. Brux. 1910 1: 105.)

It is to be regretted that this recommendation was not made retroactive. I feel confident that the retroactive fixation of nomenclatural types is a fundamental necessity in stabilizing nomenclature. I feel confident also that this aspect of the type concept will appeal more and more strongly to the followers of the Vienna Code as its advantages are recognized, especially as there is nothing in the concept that is contrary to the principles of that code. One must carefully distinguish between the concept itself and the rules for its application. The American Code has recognized the principle of types and has also formulated rules for type fixation. One may accept the principle and reject these particular rules.

The congress which adopted the Vienna Code appears to have been actuated by a desire to formulate rules that should, in a general way, preserve the current usage of generic names. I wish to point out to the followers of the Vienna Code that this laudable purpose can be accomplished with greater definiteness by applying the type concept than by applying the vague and uncertain rules adopted by the Vienna Congress.

The Vienna Code contains the following rule:

> ART. 45. When a genus is divided into two or more genera, the name must be kept and given to one of the principal divisions. If the genus contains a section or some other

---

[2] Formulated in 1907 by a Nomenclature Commission of the Botanical Club of the American Association for the Advancement of Science.

> division which, judging by its name or its species, is the type or the origin of the group, the name is reserved for that part of it. If there is no such section or subdivision, but one of the parts detached contains a great many more species than the others, the name is reserved for that part of it.

Let us apply this rule to the Linnaean genus Panicum. There are 20 original Linnaean species. Several of them, including *P. miliaceum* and its allies, belong to the genus Panicum as delimited by most modern botanists. Among the 20 are also *P. italicum* and its allies, now generally distinguished as Setaria or Chaetochloa. But *Panicum italicum* is the historic type of Panicum, that is, the species which was known as Panicum by pre-Linnaean authors and the one which I should interpret as, "judging from its name or its species, is the type or the origin of the group," and therefore the segregated genus containing it should have retained the name Panicum. However, in the process of taxonomic and nomenclatural development of the various species involved, this procedure was not followed. If botanists wish to retain the name for the allies of *Panicum miliaceum*, the simplest method to insure this result is to select *Panicum miliaceum* as the type of Panicum.

The Linnaean genus Holcus, presenting certain complications, illustrates the advantage of the type method. The name in pre-Linnaean literature was applied to the sorghums, but in the *Species Plantarum* Linnaeus unites with the three species of the sorghum group four other species of diverse relationships, one of which is *Holcus lanatus*, the only one of the species belonging to Holcus as now recognized by European botanists. The Vienna Code provides (Art. 19) that

> It is agreed to associate genera, the names of which appear in this work [*Species Plantarum*] with the descriptions of them in the *Genera Plantarum* ed. 5 (1754).

According to the Vienna Code (as well as to the American and Type-basis codes) the name Holcus should be applied to the sorghums and this I have done, since the author's concept is most accurately interpreted by his own description. But when the aggregate included under Holcus by Linnaeus in 1753 was divided, a century or more ago, the sorghums and species of other genera were taken out and the name Holcus was left for *H. lanatus*, which until recently has generally borne that name. The followers of the Vienna Code have accepted current usage regardless of the rules of that code. Would it not be simpler and more definite to make an exception and to crystallize current usage by fixing *Holcus lanatus* as the type of Holcus?

Examples could be multiplied indefinitely. Apparently the rules of the Vienna Code were left indefinite in order that botanists should not be too much restricted in the application of names and should have some freedom to use personal judgment. It is impossible to foresee all contingencies and to provide for them by definite rules. As shown above, when, in particular cases, the rules lead in the wrong direction they are likely to be ignored. The desired results can be accomplished with much greater precision by using the type method. I commend to the followers of the Vienna Code the proposal that the International Rules be modified by a recommendation to the effect that the application of names be fixed by means of nomenclatural types, this to apply retroactively.

The American Code provides for fixing the application of names by means of types. It goes further and provides rules for determining the type. It should be emphasized that the acceptance of the concept of types does not involve the acceptance of a particular set of rules for selecting types.

The code formulated by the Committee on Nomenclature of the Botanical Society of America is called the Type-basis Code of Nomenclature. Like the American and the Vienna codes, the rules of the Type-basis Code are founded on the principle of priority. The rules for selecting types of genera and of species are in conformity with this principle, while, as stated previously, the Vienna Code omits altogether the rules for selecting types (though *type* appears incidentally in Art. 45 as indicated above). It will be seen then that the chief difference between the Vienna Code and the new Type-basis Code is that the one ignores the subject and the other formulates rules for selecting types. If the Vienna Code could be modified to include a set of acceptable rules governing the selection of types, the most important difference between the two codes would disappear.

Attention should here be called to the fact that selecting the type of a group does not validate the name of that group. Types are selected for both valid names and synonyms. It only means that if a certain name is used it should be so applied as to include the type.

I will review briefly the proposed rules for selecting the types of genera. I will pass over certain particular cases such as those in which there was but one species in the genus as originally published, or in which the type was designated originally, and refer to the troublesome cases where there were several species included in the genus as originally published. This is true of many Linnaean genera, and the typification of these is basic so far as stability of nomenclature is concerned. There was an attempt at one time to select arbitrarily the first species as the type. This would be definite, but would often run counter to the historic development of the group and would cause so many changes in names as to introduce serious and needless confusion. The new code provides for selection by applying the rule of reason, taking into consideration all the factors in each case. In preparing a recent bulletin I found it necessary to typify over 300 grass genera. I will select a few examples from these. If the genus was used in his earlier works, *Flora Lapponica* or *Flora Suecica*, the type should be chosen from among those in the *Species Plantarum* that are cited by Linnaeus as being in one of the earlier works, since these are the species with which he was more familiar. Under Andropogon in the *Species Plantarum* Linnaeus describes 12 species. The name Andropogon was first used in the *Flora*

Leidensis where two species are described, both being included in the *Species Plantarum.* From these two *Andropogon virginicus* was chosen as the type because that species retained the name in its usual significance. The other species, *A. hirtus,* is now by many botanists referred to a different genus.

Poa L. Linnaeus describes 17 species. He first used the genus in his *Flora Lapponica.* From among the species there described *Poa pratensis* is selected as the type because that retains the name of this economic species in its usual signification.

Uniola L. Two species are described. One is referred now to Distichlis. The other is selected as the type, thus retaining the name in its current usage.

Hordeum L. Six species are described. The reference in the *Genera Plantarum* is to figure 295 in Tournefort's work, representing *Hordeum vulgare,* the common barley, which is therefore selected as the type.

Aira L. Of the 14 species described four are included in the *Flora Lapponica.* To take the first of these as the type would transfer the name Aira to what we now call Trisetum. Hence another one of the four, *A. caespitosa,* is selected in order to retain the name in its usual signification. Some botanists apply the name Aira to the last two of the 14 original species, including *A. caryophyllea,* and refer *Aira caespitosa* and its allies to Deschampsia. These two species are from southern Europe and were not included by Linnaeus in his first use of the term Aira in the *Flora Lapponica,* and hence did not represent Linnaeus's original idea of the genus.

In general, one should ascertain if possible what species or group of species an author had chiefly in mind in establishing a new genus.

The application of the type concept to species is similar. If more than one specimen is cited, one should find which one the author had chiefly in mind. This may be shown by comparison with the description, by one having been selected for an illustration, by notes on the original sheet, by the specific name. Only when other methods fail should the first specimen cited be arbitrarily selected.

The above illustrates what is meant by applying the rule of reason in the selection of types. Let us hope that soon all taxonomic botanists will accept the concept of types and that they may agree on the types to be selected.

Bureau of Plant Industry,
Washington, D. C.

THE RELATION OF CERTAIN NUTRITIVE ELEMENTS TO THE COMPOSITION OF THE OAT PLANT

JAMES GEERE DICKSON

(Received for publication January 8, 1921)

The study of the relation of various environmental factors to the composition of plants received its greatest stimulus when Emil Wolff published his studies on the analysis of plant ash to determine what constituents were to be found therein. Since that time an enormous quantity of literature has been contributed to the study, yet the work has never been satisfactorily concluded. Climate, availability of nutrients, water supply, and various other physico-chemical factors influence the composition of the straw greatly and of the grain or reproductive parts to a lesser extent.

W. Wolff (1864, 1865) and Hellriegel (1869) reported the first extensive study on the relation of mineral salts to plant composition. Not, however, until the voluminous work of E. Wolff (1871), did the study receive the attention of many of the best chemists in Germany. This pioneer study stimulated research until the investigations were taken up from several rather different yet related lines.

Von Heinrich (1882), Atterberg (1886, 1887), Dikow (1891), Helmkampf (1892), Stahl-Schröder (1904), Jakouchkine (1915), and Sawine (1916) sought by analysis of the whole plant or of its several parts to determine the availability of the mineral nutrients in the soil. On the other hand, Lawes and Gilbert (1856, 1884), Pagnoul (1875), and more recently LeClerc and Leavitt (1910), Raymond and Paturel (1910), Hartwell and Wessels (1913 *a*, *b*), Tretiakov (1913), Headden (1916 *a*, *b*), Davidson and LeClerc (1917), and Maschhaupt (1918) have studied the relation of environmental factors, chiefly fertility and climate, to the composition of the whole plant and of its respective parts. Griffiths (1884), Takeuchi (1908), Chirikov (1914), and Waynick (1918) have extended the investigations still farther by studying the effects of the addition of specific substances, in many cases in varying amounts, to the composition of the plant. Kossowitsch (1909) has taken still another phase of the problem, investigating the composition of different plants grown under the same nutritive conditions.

The work referred to up to this time has been primarily a study of the influence of these factors upon the composition of the ash constituents. Although it is not within the scope of this paper to discuss the relation of environmental factors to the organic composition of the plant, yet the study would not be complete without reference to the important literature on this phase of the investigation. Thacher (1913, 1917), Grisdale (1913), Tretiakov (1913), Headden (1916, *a*, *b*), and others have studied the relation of nutrition and climate to the protein composition of plants. Wiley (1901) and Wilfarth and Wimmer (1903) have shown that fertilizers and climate cause a marked variation in the sugar content of the sugar beet. Seissl and Gross (1902) state that the starch content of the potato is changed quite markedly by the addition of fertilizers. Parrozzani (1908) and Jakouchkine (1915) find considerable variation in the organically combined phosphorus of plants when different fertilizers are employed. Garner (1914) and others have shown that the oil content of plants is modified by the addition of certain fertilizer elements to the soil.

A review of this literature on the relation of plant environment to composition brings out two very striking facts: first, that the plant as a whole responds quite markedly to environment by changes in its composition, and second, that for no two cases are these responses the same. Most of the work on the relation of fertilizers to plant composition has been done in the field where sufficient quantities of most of the elements have been present to supply the minimum needs of the plant; therefore, the changes in composition, especially in that of the seed, have not been very marked. The conclusion has thus been drawn that the composition of the grain, the reproductive part, is very constant, while the response or change in plant constituents takes place in the leaves and stems of the plants.

It has been the purpose of the experiments herein recorded to study the effect of limiting certain essential nutrient elements upon the composition of the grain and straw of well matured plants when other environmental factors were controlled as far as possible.

The culture work from May to August, 1915, and May to August, 1916, was carried on under climatic conditions different from those that governed the latter part of the work, which differences may in some cases explain the differences between the data recorded for the two respective periods. A brief comparison of the meteorological data for the two periods has been included in the present paper.

From September, 1916, until the conclusion of the experiments the work has been carried on in the laboratory of plant physiology at the University of Wisconsin. It is a pleasure to acknowledge my indebtedness to Professor J. B. Overton for his advice and assistance in supplying the apparatus necessary during the course of the investigation. Acknowledgment is also gladly given Professor W. E. Tottingham for his advice and assistance in the analytical work necessary during the progress of the experiments.

METHODS

Cultural methods employed in growing the plants under varied nutritive conditions are given in detail in a previous paper (Dickson, 1918). In brief, the complete cultural series consisted of the "normal" solution—that is, Knop's solution—modified by the addition of 0.1 gram of sodium chloride per liter of solution, and diluted to one tenth the concentration usually listed (table 1); and of five further modified culture solutions in each of which one of the elements magnesium, calcium, potassium, phosphorus, and nitrogen was reduced to one tenth of the quantity present in the normal solution.

TABLE 1. *The Composition of the Modified Knop's Solution Used as the Normal Culture Solution*

Salts	Concentration of Salts Used in "Normal" Solution Grams per Liter of Solution	 Percentage Composition
$Ca(NO_3)_2$	0.08	0.0533
KNO_3	0.02	0.0133
KH_2PO_4	0.02	0.0133
$MgSO_4$	0.02	0.0133
NaCl	0.01	0.0067
Total salt	0.15	0.1000

Although these deficient elements were reduced to such an extent that they seriously hindered the development of the plant, yet they did not in any case prevent the production of grain. In other words, the various nutritive elements were reduced to about the lowest concentration that would still support the production of grain by the plants growing therein. It was necessary, of course, to base the concentration of the element reduced in each solution on the constituents in greatest demand by the plant, namely, phosphorus and nitrogen, and then to prepare all of the solutions in which one of the essential nutritive elements is deficient with the same concentration of the deficient element. By reducing the amount of the various nutritive elements below that required for normal development, it was hoped to bring out more strikingly the relation of the elements to the composition of the plant.

The various cultural solutions were calculated and prepared upon the basis of equal osmotic concentration by varying to a slight degree the amount of sodium chloride added to the respective solutions. To state it differently, the required nutritive salts were added to the solution and their respective osmotic activity was calculated from the dissociation of each constituent salt, then sufficient sodium chloride was added—never enough to exert a toxic influence—to make the total osmotic concentration equal to that of the "normal" solution. It was not found practicable to take into account the effect of one constituent salt upon the dissociation of another in the culture solution, but the degree of dissociation of the various solutions was checked by the soluble salts present, by measurements of electrical conductivity, and by freezing-point determinations.

The quantity of sodium chloride used in balancing the modified nutritive solutions was in no case greater than that added to the normal solution, that is, 0.0067 percent of the total salts added (table 1). Therefore, the presence of this salt should have no appreciable difference in effect upon the

TABLE 2. *The Amounts of Salts in Solution, Electrical Conductivity, and Depression of the Freezing Point of the Normal and Modified Solutions*

Solution, Deficient Element Given	Total Soluble Salts in Grams per 100 Cc. of Solution			Conductivity × 10^{-5} Reciprocal Ohms			Depression of Freezing Point in Degrees Centigrade		
	1916 Sol.	1917 Sol.	Ave.	1916 Sol.	1917 Sol.	Ave.	1916 Sol.	1917 Sol.	Ave.
Normal..................	.110	.105	.107	1.363	1.507	1.435	.050	.051	.050
Mg 0.1..................		.105			1.556			.051	
Ca 0.1..................	.085	.070	.077	1.144	1.058	1.101	.050	.050	.050
K 0.1..................	.109	.082	.095	1.343	1.451	1.397	.050	.051	.050
P 0.1..................	.098	.090	.094	1.383	1.305	1.344	.050	.051	.050
N 0.1..................	.120	.109	.114	1.455	1.625	1.540	.050	.061	.055

development or composition of the plants produced in the respective solutions.

General Cultural Methods

Glazed earthenware jars were used for containers. The plants were grown in analyzed quartz sand to which was added the diluted nutritive solution. The solution in the culture jars was maintained as nearly as possible at 60 percent of the moisture-holding capacity of the sand.

Pedigreed Swedish select oats, *Avena sativa aristata*, were used in all the experiments. The plants were grown under an open glass house exposed to out-of-door conditions from May until August of each year. The cultural work from May to August, 1915, and from May to August, 1916, was carried on under climatic conditions different from those that governed the latter part of the work, which differences may in some cases explain the differences between the data recorded for the two respective periods. In a later part of this paper a comparison will be made of the meteorological conditions for the two periods.

The average grain and straw produced per pot for each year is given in table 3. It was not possible to produce large quantities of either grain or straw in the phosphorus- and nitrogen-deficient solutions. Therefore, the samples for analysis from these cultures were rather small, yet with special manipulation it was possible to secure results that checked very closely.

TABLE 3. *The Average Yield of Grain and Straw in Plants Grown in the Normal Solution and in Solutions with One Nutrient Element in Each Case Reduced to One Tenth Normal*

No. Det. per Year	Solution, Deficient Element Given	Weight of Grain in Grams			Weight of Straw in Grams		
		1915 Crop	1916 Crop	1917 Crop	1915 Crop	1916 Crop	1917 Crop
4.....	Normal	5.329±2	6.986±1	6.362±0	7.08±2	14.77±2	28.08±0
2.....	Mg 0.1			5.804±1			29.62±2
2.....	Ca 0.1	5.212±1	6.645±0	3.999±0	7.96±2	17.21±3	20.75±2
2.....	K 0.1	2.968±1	5.660±2	3.268±0	3.51±2	10.22±4	12.68±0
2.....	P 0.1	1.142±1	.390±1	.665±0	1.59±1	.84±1	2.11±2
2.....	N 0.1	1.767±1	.120±1	.679±0	2.23±1	.29±0	2.02±0

In most cases, the quantity of plant material produced was too small to permit the analysis of the grain and straw for all the essential nutrient elements limited in the cultural series; therefore, it was necessary to select certain of these plant constituents which would represent elements having a very definite chemical combination with many of the complex plant substances, and certain others whose action was more or less secondary in nature. Phosphorus and calcium were finally selected, and determinations were made for total phosphorus and calcium present in the oat grain and straw.

Analytical Methods

The well matured grain and straw were ground to a finely powdered mass after drying at 90° C. for 12 hours, and stored in tightly stoppered bottles for analysis. After the samples were dried to constant weight at 110° C. and thoroughly mixed, samples were weighed out for calcium and phosphorus determinations. Two-gram samples for grain and one-gram samples for straw were taken for the calcium determinations, and one-gram samples of both grain and straw were used for phosphorus determinations.

The official analytical methods as set forth in Bureau of Chemistry Bulletin 107, or standard methods which had been carefully checked with the "official methods," were employed in all cases.

All analyses were run in duplicate, and, in case they did not check within 0.5 percent, were repeated. In the cases of three samples, however, of which there was insufficient material, only one sample was analyzed. The results given in the tables are averages of these duplicate, or in some cases quadruplicate, determinations.

The samples for calcium determinations were ignited at low heat in a muffle until completely ashed, digested in hydrochloric acid, and finally filtered. Calcium was precipitated as calcium oxalate after removing the hydroxides of iron, aluminum, and phosphorus, and determined as CaO by titrating the oxalic acid with standard potassium permanganate.

The phosphorus samples were analyzed by the sodium-peroxide method as originally described by Osborne (1902) and modified by Dubois (1905). The sample was placed in a nickel crucible and moistened until it formed a thick paste, after which five grams of anhydrous sodium carbonate were added and the charge was mixed immediately. Five grams of sodium peroxide were then added in smaller portions at a time with thorough mixing after each addition. The mass was then heated until fusion was complete. Additional sodium peroxide was added to oxidize completely the organic matter. The mass was dissolved in concentrated hydrochloric acid, made up to 250-cc. and 100-cc. aliquot portions used for phosphorus determinations. These 100-cc. samples were evaporated to dryness over a water bath, taken up with hot water, strongly acidified with nitric acid, and phosphorus was determined by the "official gravimetric method."

THE RELATION BETWEEN NUTRIENTS AND CALCIUM CONTENT OF GRAIN

The general conception has been that there is very little variation in the composition of the seeds or reproductive parts of plants. Lawes and Gilbert (1884) state that the composition of the grain is not greatly varied by normal variations in soil composition. Their ideas of the limits of variation of the grain can best be stated in their own words:

> The composition of the grain only varies in any marked degree according to manure, when there is a very abnormal deficiency of one or more constituents, having regard to the amount of growth which is induced by the liberal supply of others. The composition of the grain is very uniform, notwithstanding there may be a very great excess of supply, and a relatively very great excess taken up by the plant, in which latter case a large excess remains in the straw.

As previously stated, the earlier studies on composition have usually been made on plants which were grown under field conditions in soil of low or high fertility, or, in other words, where the supply of the available nutritive elements was not under strict limitations. It is evident from the data presented in table 4 that the calcium content of the grain varies greatly when the supply of available nutritive elements is limited to a definite small amount.

TABLE 4. *The Average Calcium Content of Grain from Plants Grown in the Normal Solution and in Solutions with one Nutrient Element in Each Case Reduced to One Tenth Normal*

Solution, Deficient Element Given	Percent CaO in Grain			
	1915 Crop	1916 Crop	1917 Crop	Average
Normal	0.317	0.405	1.360	0.694
Ca 0.1	0.042	0.061	0.180	0.094
Mg 0.1			1.422	0.729
K 0.1	0.216	0.227	1.760	0.734
P 0.1	0.275	0.284	0.540	0.367
N 0.1	0.305	0.548	0.500	0.451

The plants grown in culture solutions deficient in calcium—one tenth the amount in the normal culture solution—produced grain of very low calcium content, an average of ten percent lower than the calcium content of the grain produced in the normal solutions.

Physiologists and biochemists consider the rôle of calcium as secondary in the formation of seed. Therefore, possibly it may be replaced to a greater or less extent by certain other bases, notably potassium and magnesium. Some proof supporting this hypothesis is given in the fact that the average calcium content of the grain produced in magnesium-deficient and potassium-deficient solutions is relatively high. The low calcium content of the grain produced in potassium-deficient solutions during the first two years, however, would indicate that this is not true under all environmental conditions. The calcium content of the grain produced in the phosphorus- and nitrogen-deficient solutions during the first two seasons is quite high.

These samples were rather small, however, and therefore the data are not as accurate as desired. The calcium content of the grain from the phosphorus- and nitrogen-deficient solutions for the last year (1917 column, table 4) in which the total number of plants was greatly increased, making more material available for analysis, is considerably lower than in any of the other samples. The data for these last two series, namely, phosphorus- and nitrogen-deficient cultures, are too varied to draw any conclusions. In the case of the other samples, however, it is quite evident that a marked variation in the nutritive solution produces a very considerable change in the calcium content of the grain.

It is held by some that calcium and magnesium function in the translocation of carbohydrates and proteins and in the storage of these compounds during seed formation, rather than being directly connected with the synthesis of the carbohydrates and proteins that are later transferred to the seed. Calcium probably functions in this capacity much less than does magnesium, as analyses show a scarcity of calcium and an abundance of magnesium in most seeds. Magnesium probably functions, therefore, as the chief carrier of phosphoric and other acids entering into the chemical composition of the seed, while calcium acts more as a neutralizer of acids resulting from synthesis. Bernardini (1914) describes quite fully the functions of magnesium and its probable relation to translocation processes. This indirect function of calcium was first pointed out by Holzner (1867) and Schimper (1890), and more recently.has been supported by Chirikov (1914), Robert (1917) and others.

Truog (1916) states that plants with a high protein content generally have a high calcium content and that when manganese phosphate is used instead of calcium phosphate as a source of phosphorus the plants grown in such a solution have an extraordinarily high manganese content. Robert (1911, 1912) attempts to show that calcium is deposited directly within the fungus as a calcium salt of certain organic acids. She shows that an increase of calcium in the culture solutions results in a very marked rise in the calcium content of the fungus.

In general, a deficiency of calcium in the nutritive solution results in the production of grain with a very low calcium content, while on the other hand a deficiency of magnesium or of potassium in the culture solution causes a slight accumulation of calcium in the grain of the plants grown therein. The effect of a deficiency of either phosphorus or nitrogen generally results in the production of grain with a low calcium content. The calcium content of the grain of oat plants grown under varied nutrient conditions is considerably altered by the composition of the nutrient solutions in which the plants grow.

The Relation between Nutrients and Calcium Content of Straw

The calcium content of the straw of the oat plant varies in a way in general similar to, although more marked than, that characteristic of the grain. Lawes and Gilbert (1884) have shown that the composition of the straw (leaves and stems) of various plants may be modified very markedly by the addition of nutritive elements as well as by the subtraction of these elements. The results have been so consistent in field work of this nature as to lead certain investigators to suggest that the composition of the grain, the reproductive part of the plant, is very stable, while the variation, if any occurs, is in the straw and roots. The average percentages of calcium in the straw produced in the different culture solutions are given in table 5.

Table 5. *The Average Calcium Content of Straw from Plants Grown in the Normal Solution and in Solutions with One Nutritive Element in Each Case Reduced to One Tenth Normal*

Solution, Deficient Element Given	Percent CaO in Straw			
	1915 Crop	1916 Crop	1917 Crop	Average
Normal	2.315	2.176	4.850	3.114
Ca 0.1	0.520	0.220	0.485	0.408
Mg 0.1			4.030	2.589
K 0.1	2.362	1.666	4.260	2.763
P 0.1	3.175	1.072	0.935	1.727
N 0.1	1.288	1.335	1.935	1.519

The average calcium content of the straw from the calcium-deficient solutions—containing one tenth of the calcium present in the normal solution —is only 13 percent of that of the straw produced in the normal cultures. The calcium content of the straw from the potassium-deficient solutions, although high comparatively speaking, is not as high as in the grain from the same set of cultures. The calcium content of the straw of the plants from the phosphorus-deficient cultures is considerably lower than that of the plants from the nitrogen-deficient solutions, and in both it is lower than the calcium content of the check plants.

In general, a deficiency of calcium in the culture solution causes a very marked lowering of the calcium content of both grain and straw of plants grown therein. A deficiency of potassium or of magnesium does not greatly affect the intake and storage of calcium either in the grain or in the straw. A deficiency of either phosphorus or nitrogen causes a lowering of the calcium content of both grain and straw; especially is this true for plants in the phosphorus-deficient solutions. The average composition of the plants for the three years is given in table 8, and in graphic form in figure 1.

The Relation between Nutrients and Phosphorus Content of Grain

Phosphorus, unlike calcium, enters into chemical combination with a great many of the plant compounds, especially with those of the seed.

Phospholipins, nuclein and nucleic acid, phytin, and possibly starch contain phosphorus in chemical combination. In addition to these organic compounds, some inorganically combined phosphates are stored in the seed, and often large quantities are deposited in the straw of the cereals. Probably, lecithin and phytin are the only organic compounds containing phosphorus which are accumulated as reserves in the seed, and which are, therefore, subject to variation due to the supply of phosphorus. Parrozzani (1908) found that the percentages of both lecithin and phytin phosphorus were increased by the addition of mineral phosphate fertilizers to the soil. The nuclein phosphorus, on the other hand, was quite constant, showing no change even with the most varied phosphorus fertilization. Jakouchkine (1915) stated that the amount of phytin in the grain is apparently dependent on the condition of the soil. Generally only small amounts of inorganically combined phosphorus are stored in the grain; therefore, if the total phosphorus of the grain varies with varying amounts in the soil, it is probably due to the varying amounts of the organic reserve materials containing phosphorus. An organic analysis is necessary to disclose these relations and is planned in further pursuit of this problem.

The composition of the grain from plants grown in culture solutions having a deficiency of certain nutrient elements, especially of phosphorus, is modified very markedly. The average percentages of total phosphorus in the grain from plants grown in the different culture solutions are given in table 6.

TABLE 6. *The Average Phosphorus Content of Grain from Plants Grown in the Normal Solution, and in Solutions with One Nutrient Element in Each Case Reduced to One Tenth the Normal Amount*

Solution, Deficient Element Given	Percent P_2O_5 in Grain			
	1915 Crop	1916 Crop	1917 Crop	Average
Normal	0.673	1.047	1.900	1.206
Ca 0.1	0.946	1.017	2.130	1.364
Mg 0.1			1.720	1.092
K 0.1	0.625	0.963	1.665	1.084
P 0.1	0.472	0.732	0.453	0.552
N 0.1		1.121	1.552	1.336

The phosphorus composition of the check plants which were grown in a complete nutrient solution varies over the three years' experiments. These variations for the different years will be discussed later under another heading. The phosphorus content of the grain from the plants grown in the calcium-deficient solutions is the highest of any of the series, the average content for the three years being 0.158 percent higher than the composition of the grain from the checks. The explanation for this extra high phosphorus content may be sought from two different sources: first, the calcium-magnesium ratio of the Knop's solution may not be the best for maximum translocation of phosphates, if, as is thought by a number of biochemists, magnesium is the chief carrier of phosphoric acid; and second, the calcium in the normal Knop's solution may be present in sufficient quantity to react with the phosphates to form the less soluble tricalcium phosphate, thus making the phosphorus present less available for the plant. The nitrogen-deficient culture solutions, likewise, produced plants with grain having a very high total phosphorus content. In all probability the factors concerned here are very complicated, as the deficiency of proteins would be apt to upset normal metabolism and therefore to produce very abnormal results within the plant. It is quite possible that much of the phosphorus in this latter case may be stored as the mineral phosphate in combination with the large amounts of bases present. The nutrition of the plant in this case is undoubtedly very much disturbed and very abnormal. A deficiency of potassium in the culture solution, on the contrary, results in the production of grain with a low phosphorus content, the average phosphorus content for the three years being 0.122 percent lower than in the check. The greatest reduction in phosphorus content, however, is in the grain from phosphorus-deficient solutions. The phosphorus content of this grain is reduced to 46 percent of the phosphorus present in the grain from the checks. In all probability the reduction took place in the amount of phytin and lecithin stored in the grain. In general, then, there is a very marked variation in the phosphorus content of grain produced under different nutritive conditions. A deficiency of phosphorus or of calcium causes the greatest variation, the lack of the former element producing a very marked decrease, that of the latter a moderate increase in phosphorus content.

THE RELATION BETWEEN NUTRIENTS AND PHOSPHORUS CONTENT OF STRAW

The composition of the straw generally shows more marked variations than that of any other part of the plant, unless it be the roots, for the unassimilated excess salts are stored here in the case of the addition of large amounts of specific elements, and on the other hand the straw releases

TABLE 7. *The Average Phosphorus Content of Straw from Plants Grown in the Normal Solution and in Solutions with One Nutrient Element in Each Case Reduced to One Tenth the Normal Amount*

Solution, Deficient Element Given	Percent P_2O_5 in Straw			
	1915 Crop	1916 Crop	1917 Crop	Average
Normal	0.309	0.734	0.832	0.628
Ca 0.1	0.415	0.354	1.230	0.667
Mg 0.1			0.892	0.673
K 0.1	0.265	0.334	0.682	0.427
P 0.1	0.092	0.060	0.051	0.068
N 0.1	0.834	1.750	1.805	1.463

the deficient substances quite rapidly in order that they may be stored in the reproductive parts of the plant. A wider variation in the composition of the straw is, therefore, to be expected. The results, as set forth in table 7, show this to be true. The variations in composition due to varied nutritive conditions are similar to, though more marked than, those of the grain. The extremely high phosphorus content of the straw from the nitrogen-deficient solutions confirms the idea that the phosphorus metabolism is blocked by the deficiency of proteins, and that, therefore, phosphorus in the inorganic form piles up to some extent in the grain, but more markedly in the straw. The phosphorus content of the straw produced in the phosphorus-deficient culture solutions is very low, the average for the three years being approximately 10 percent of the amount present in the straw from the normal solutions. The variations in the composition, although more pronounced in the straw, yet in general are identical with those in the grain.

TABLE 8. *The Average Composition of Plants Grown in the Normal Solution and in Solutions with One Nutrient Element in Each Case Reduced to One Tenth the Normal Amount*

Solution, Deficient Element Given	Percent CaO		Percent P_2O_5	
	Grain	Straw	Grain	Straw
Normal	0.697	3.114	1.206	0.628
Ca 0.1	0.097	0.408	1.364	0.667
Mg 0.1	0.729	2.589	1.092	0.673
K 0.1	0.734	2.763	1.084	0.427
P 0.1	0.366	1.727	0.552	0.068
N 0.1	0.451	1.519	1.336	1.463

In summation, comparing the composition of the plants from the modified solutions with the composition of those from the normal, a deficiency of phosphorus in the culture solution causes a very marked lowering of the phosphorus content of both grain and straw of the plants grown in these solutions. A deficiency of either calcium or nitrogen causes an increased intake of phosphorus especially in the straw. A deficiency of potassium causes a slight reduction in the amount of phosphorus accumulated in the grain and a more marked reduction of phosphorus in the straw. Magnesium deficiency in the culture solutions causes a slight decrease of phosphorus in the grain of plants grown in these solutions, and a small increase in the phosphorus content of the straw. The average composition of the plants for the three years is given in table 8, and in graphic form in figure 1.

THE RELATION OF CLIMATE TO THE COMPOSITION OF THE OAT PLANT

The experiments of Lawes and Gilbert (1884), Cserhati (1908), Grisdale (1913), and Tretiakov (1913) have shown very strikingly that the factors of climate and geographical location cause a wide variation in the composition of plants. Lawes and Gilbert (1884) explain these variations by deviations from the normal maturation of the plant. They sum up their results very well in the following statements:

> The character of the crop left to ripen depends very much more upon season than upon manuring. There is scarcely any difference in the composition of the truly and normally ripened seed. The wide range in the composition of the ash of the grain represents a corresponding deviation from the normal development.

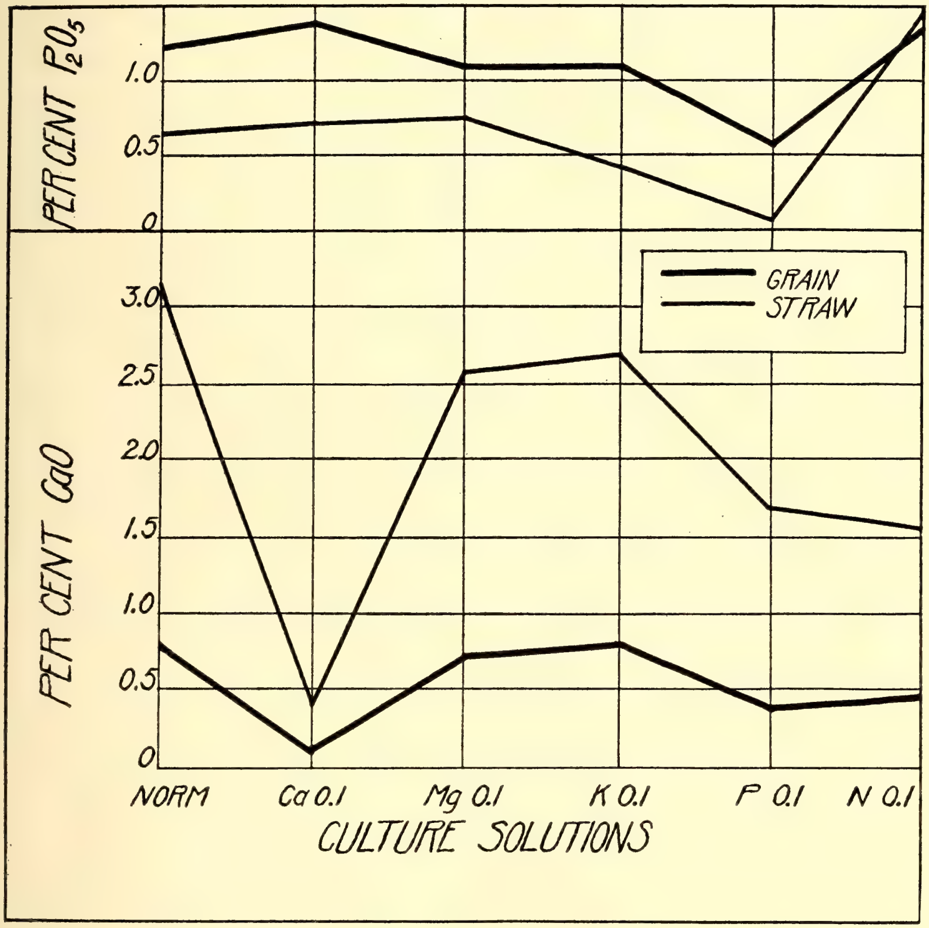


FIG. 1. Curves showing the average composition of grain and straw of plants grown in the normal solution and in solutions with one nutrient element in each case reduced to one tenth the normal amount.

Likewise LeClerc and Leavitt (1910) attribute the variations in composition of wheat primarily to different climatic conditions and to the effect of these climatic factors upon plant growth and maturation. It is undoubtedly true that environmental factors which alter normal development and maturation have an extremely great influence upon the composition of crops grown in the field or in artificial culture. In artificial cultures, however, it is possible to alter the growth and maturation of the plant much more by the deficient nutrients than by climatic factors; therefore, it is reasonable to suppose that the deficient nutrient may have as much influence upon the composition as the climatic variations. This is not, however, minimizing the effect of climate, and such environmental factors must be taken into consideration in all studies of this sort whether they be field or culture experiments.

The analytical data previously discussed in connection with nutrition, when studied from another angle, that of the relation of climate and geographic location to composition, show how closely the influences of these various factors are interrelated. The combination of temperature, humidity, and other climatic conditions greatly influenced the composition of the plants except when the reduction of certain essential nutritive elements became the predominant factor in controlling plant development. Under these conditions, variations due to other environing factors were outweighed by the deficient nutritive element.

TABLE 9. *The Average Composition of Plants grown under Different Climatic Conditions*

	Composition of Plants in Percentage Based on Dry Weight		
	Pullman, Washington		Madison, Wisconsin
	1915	1916	1917
Average composition from all cultures			
CaO content of grain	0.231	0.305	0.960
P_2O_5 content of grain	0.679	0.976	1.570
CaO content of straw	1.932	1.294	2.416
P_2O_5 content of straw	0.383	0.646	0.915
Composition of check plants			
CaO content of grain	0.317	0.405	1.360
P_2O_5 content of grain	2.315	2.176	4.850
CaO content of straw	0.673	1.047	1.900
P_2O_5 content of straw	0.309	0.734	0.832
Composition of plants grown in Ca-deficient solution			
CaO content of grain	0.042	0.061	0.180
CaO content of straw	0.520	0.220	0.485
Composition of plants grown in P-deficient solution			
P_2O_5 content of grain	0.472	0.732	0.552
P_2O_5 content of straw	0.092	0.060	0.051

The analysis of plants grown in 1915 and in 1916 at Pullman, Washington, show in general a smaller accumulation of both calcium and phosphorus than in the last year, 1917, in which the plants were grown at Madison, Wisconsin. The phosphorus content of both grain and straw of plants grown in the complete nutrient solution and in the solutions with one nutritive element in each case reduced to one tenth that in the control solution, with the exception of the phosphorus-deficient solution, show consistent variations for the three years. With few exceptions, the phosphorus content is highest in the 1917 samples and lowest in the 1915 samples.

The average phosphorus content of all the grain analyzed each year, as given in table 9, is: 0.679 percent in 1915, 0.976 percent in 1916, and 1.570 percent in 1917. The increases in phosphorus content of the straw are equally great. This stands out in extreme contrast with the phosphorus content of the grain and straw of the plants grown in the phosphorus-deficient solutions, which show no influence of climate on composition. The calcium content of the grain produced in all the cultures, including the calcium-deficient solutions, is highest in 1917 and lowest in 1915. The calcium content of the straw, on the other hand, is not greatly influenced by seasonal differences. Certain other differences are brought out in comparing the data presented in tables 4 and 7 and summarized in table 9, but before they can be discussed intelligently a more complete analytical study is necessary together with more detailed meteorological records.

TABLE 10. *The Mean Daily and the Average Monthly Air Temperatures at Pullman, Washington, and Madison, Wisconsin, for the Duration of the Cultural Experiments*

Days	Mean Daily Air Temperature by Months											
	May			June			July			August		
	Pullman		Madison	Pullman		Madison	Pullman		Madison	Pullman		Madison
	1915	1916	1917	1915	1916	1917	1915	1916	1917	1915	1916	1917
1......	38	52	42	56	51	53	70	57	69	74	64	72
2......	45	54	42	44	52	48	72	53	61	80	63	68
3......	51	55	46	57	52	60	74	51	59	70	63	68
4......	55	—	38	63	57	61	72	55	64	69	61	72
5......	58	—	40	65	46	54	62	60	68	76	62	73
6......	58	51	44	62	52	59	66	62	66	70	67	68
7......	60	38	48	64	56	54	63	69	70	71	67	68
8......	60	42	48	55	60	61	60	73	75	70	60	62
9......	55	37	46	53	52	64	62	67	69	75	62	62
10......	48	37	50	49	49	68	60	61	66	77	66	64
11......	53	40	48	48	51	67	66	68	65	76	67	70
12......	51	44	50	54	57	71	65	74	65	71	70	64
13......	52	43	50	59	61	61	57	63	67	72	69	65
14......	51	46	57	61	65	50	53	64	68	76	71	68
15......	49	51	68	62	69	52	58	71	70	77	64	72
16......	58	56	68	61	73	56	63	69	66			
17......	55	58	70	63	76	66	56	52	68			
18......	49	51	69	51	60	71	63	54	70			
19......	47	47	68	53	47	64	69	62	74			
20......	50	45	50	60	45	64	73	66	76			
21......	54	42	42	63	42	67	82	66	78			
22......	50	52	40	70	54	59	79	65	74			
23......	52	53	44	72	61	59	72	64	76			
24......	54	54	52	65	60	61	74	66	76			
25......	52	59	56	59	66	68	72	60	80			
26......	53	53	51	54	58	73	69	55	81			
27......	61	55	52	58	59	66	66	61	75			
28......	57	50	54	62	49	60	66	61	78			
29......	51	48	60	59	50	68	56	64	85			
30......	55	55	60	63	55	70	65	71	86			
31......	62	50	62	—	—	—	68	71	84			
Average...	53	49	52	59	56	62	66	63	72	74	65	69

TABLE 11. *The Average Monthly Air Temperature at Pullman, Washington, and Madison, Wisconsin, for the Duration of the Cultural Experiments*

Month	Temperature °F. at Pullman, Washington		Temperature °F. at Madison, Wisconsin
	1915	1916	1917
May	53	49	52
June	59	56	62
July	66	63	72
August*	74	65	68

* The August temperatures are from the first to the fifteenth inclusive.

TABLE 12. *The Average Monthly Precipitation at Pullman, Washington, and Madison, Wisconsin, for the Duration of the Cultural Experiments*

Month	Rainfall in Inches at Pullman, Washington		Rainfall in Inches at Madison, Wisconsin
	1915	1916	1917
May	2.77	1.56	3.33
June	0.53	2.34	6.47
July	0.77	0.45	3.10
August	0.00	1.24	2.72

The important meteorological data for the periods during the growth and maturation of the plants are given in tables 10 and 11 and in graphic form in figure 2. The climatic differences for the three years, the first two at Pullman, Washington, and the last at Madison, Wisconsin, are summarized as follows: The 1915 growing season was very dry and cool, with high temperatures during maturation; the 1916 season was moderately dry and cool; the 1917 period was very wet and hot. The light intensity during the last year was considerably lower than during the first two seasons.

No attempt is made to correlate the variations in composition with any individual factor, for it is impossible to do more than speculate until controlled experiments have been run to determine the relation of such factors as light intensity, air and soil temperature, and humidity on the development and composition of plants.

SUMMARY

1. The calcium content of both grain and straw is reduced to about 10 percent of that of the plants from the controls by reducing the calcium in the culture solution to one tenth the quantity present in the complete nutrient solution. It is greatly reduced in both grain and straw by a similar deficiency in phosphorus or in nitrogen.

2. The total phosphorus content of the grain is reduced to 46 percent, and of the straw to 10 percent, of that in the plants from the controls by reducing the phosphate in the culture solution to one tenth of the quantity

present in the complete nutrient solution. It is slightly reduced in both grain and straw by a similar deficiency in potassium, and is increased by a similar reduction of calcium or nitrogen.

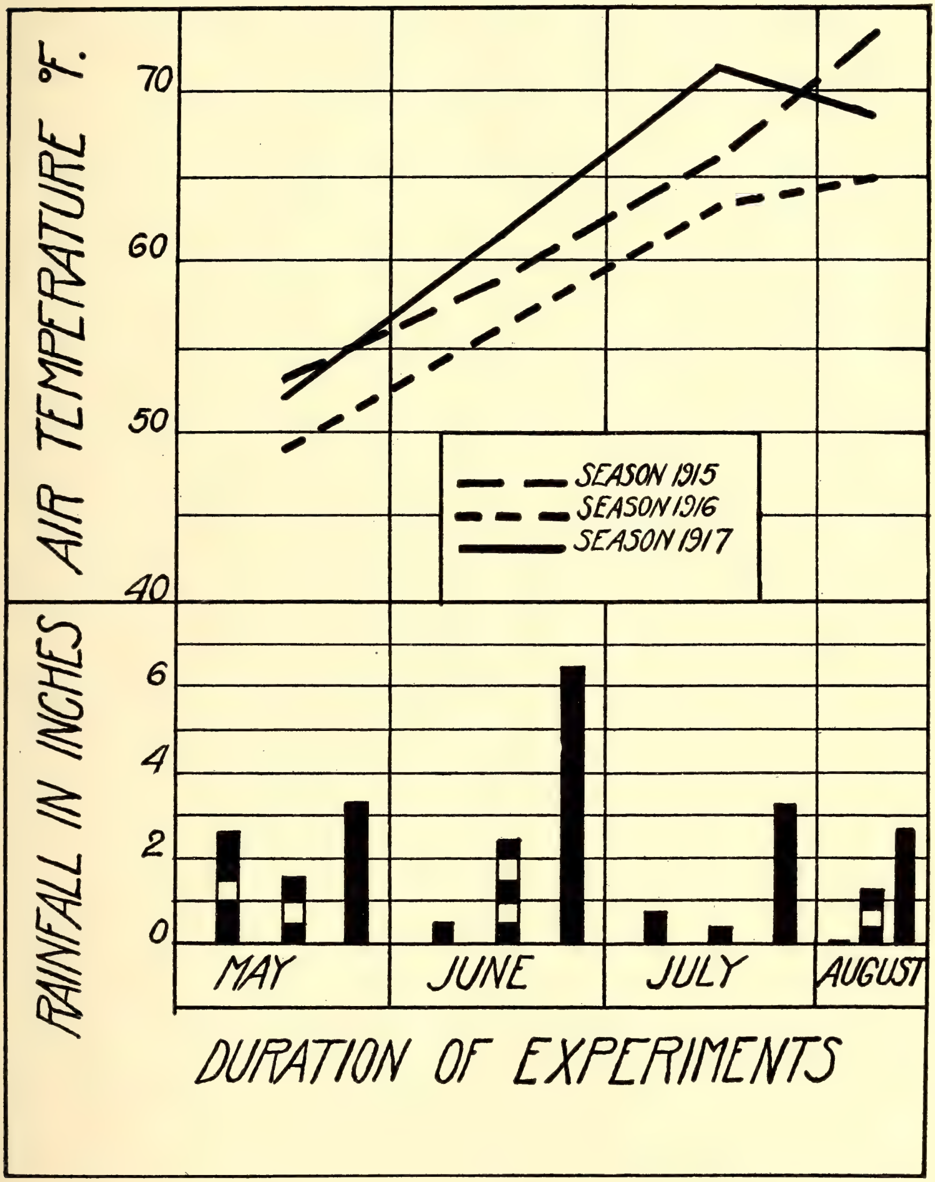


FIG. 2. Graphic representation of the average monthly temperatures and average monthly precipitation at Pullman, Washington, and Madison, Wisconsin, for the duration of the cultural experiments.

3. Although the variations in composition are more pronounced in the straw, yet in general they are similar in both grain and straw.

4. The phosphorus content of both grain and straw is modified by seasonal differences, except for the plants grown in the phosphorus-deficient solutions. The calcium content of the grain is modified by seasonal differences even in the calcium-deficient solutions. The calcium content of the straw, however, shows no consistent response to climate.

University of Wisconsin

LITERATURE CITED

Atterberg, A. 1886. Die Beurtheilung der Bodenkraft nach der Analyse der Haferpflanze. Landw. Jahrb. **15**: 415–419.

——. 1887. Die Beurtheilung der Bodenkraft nach der Analyse der Haferpflanze. Landw. Jahrb. **16**: 757–761.

Bernardini, L. 1914. Funzioni biochimiche del magnesio nella vita della pianta. Scuola Sup. Agr. Portici II, **12**: 361–389.

Chirikov, T. W. 1914. Zur Frage über die lösende Wirkung der Wurzeln. Russ. Jour. Exp. Landw. **15**: 54–65.

Cserhati, A. 1908. Die Faktoren, welche auf die Zusammensetzung des Weizens von Einfluss sind. Kiserlet. Közlem. **11**: 253–275.

Davidson, J., and LeClerc, J. A. 1917. The effect of sodium nitrate applied at different stages of growth on the yield, composition, and quality of wheat. Jour. Amer. Soc. Agron. **9**: 145–154.

Dickson, J. G. 1918. The value of certain nutritive elements in the development of the oat plant. Amer. Jour. Bot. **5**: 301–324.

Dikow, U. 1891. Beurtheilung des Bodens nach den Wurzeln der Gerstenpflanze. Jour. Landw. **39**: 134–147.

Dubois, W. L. 1905. Determination of sulphur and phosphoric acid in foods, feces and urine. Jour. Amer. Chem. Soc. **27**: 729–732.

Garner, W. W. 1914. Oil content of seeds as affected by the nutrition of the plant. Jour. Agr. Res. **3**: 227–249.

Griffiths, A. B. 1884. Experimental investigations on the value of iron sulphate as a manure for certain crops. Chem. Soc. Jour. **45**: 71–75.

Grisdale, J. H. 1913. Field crops work at the Canadian Experiment Stations and Farms in 1912. Can. Exp. Farms Repts. (1912): 47–53, 227–245.

Hartwell, B. L., and Wessels, P. H. 1913*a*. The effect of sodium manuring on the composition of plants. R. I. Agr. Exp. Sta. Bull. **153**: 1–118.

—— ——. 1913*b*. The effect of soil phosphorus on turnips as influenced by the amount available in soils. R. I. Agr. Exp. Sta. Bull. **154**: 1–148.

Headden, W. P. 1916*a*. A study of Colorado wheat II. Col. Agr. Exp. Sta. Bull. **217**: 3–46.

——. 1916*b*. A study of Colorado wheat III. Col. Agr. Exp. Sta. Bull. **219**: 3–131.

von Heinrich, R. 1882. Grundlagen zur Beurtheilung der Ackerkrune. pp. 77.

Hellriegel, F. H. 1869. Ueber die Bedeutung der chemischen Untersuchung der Ernteproducte, namentlich der Aschenanalysen, für die Beurtheilung der Menge und des gegenseitigen Verhältnisses der im Boden vorhandenen aufnehmbaren Pflanzennährstoffe. Landw. Versuchsst. **11**: 136–144.

Helmkampf, A. 1892. Untersuchungen über die Feststellung des Düngungsbedürfnisses der Ackerböden durch die Pflanzenanalyse. Jour. Landw. **40**: 85–183.

Holzner, J. 1867. Ueber die physiologische Bedeutung des oxalsäuren Kalkes. Flora **50**: 497–511.

Jakouchkine, I. 1915. Phosphore facilement extractif et suffisance de la nutrition phosphatée. Communication préliminaire. Russ. Jour. Exp. Landw. **16**: 118–139.

Kossowitsch, P. 1909. Pflanze, Phosphorit und Boden nach Versuchen des Landwirtschaftlichen Chemischen Laboratoriums zu St. Petersburg. Russ. Jour. Exp. Landw. **10**: 782–842.

Lawes, J. B., and Gilbert, J. H. 1856. The composition of the wheat grain and its products. Trans. Brit. Assoc. Adv. Sci. **26**: 173.

——— — ———. 1884. On the composition of the ash of wheat grain, and wheat straw, grown at Rothamsted, in different seasons, and by different manures. Chem. Soc. Jour. **45**: 305–407.

LeClerc, J. A., and Leavitt, S. 1910. Tri-local experiments on the influence of environment on the composition of wheat. U. S. Dept. Agr. Bur. Chem. Bull. **128**: 1–18.

Maschhaupt, J. G. 1918. De involed van grondsoort en bemesting op het gehalte onzer culture gewassen aan stikstofen aschbestauddeckn. Verslag. Landbouwk. Onderzoek. Rijkslandbouwproefstat (Netherlands) **22**: 25–116.

Osborne, T. B. 1902. Sulphur in protein bodies. Jour. Amer. Chem. Soc. **24**: 140–167.

Pagnoul, M. 1875. Sur le rôle exercé par les sels alcalins sur la végétation de la betterave et de la pomme de terre. Compt. Rend. Acad. Sci. (Paris) **80**: 1010–1014.

Parrozzani, A. 1908. Influenza di quantità progressive di concimi fosfatici sul contenuto in sostanze organiche fosforate ed azotate, e sul rapporto fra fosforo ed azoto dei semi di mais. Staz. Sperim. Agrar. Italy **41**: 729–738.

Raymond, B., and Paturel, G. 1910. De l'influence des engrais chimiques sur la composition des graines de céréales. Prog. Agr. et Vit. (Edl. Est-Centre) **53**: 777–780.

Robert, Thérèse. 1911. Influence du calcium sur le développement et la composition minérale de l'*Aspergillus niger.* Compt. Rend. Acad. Sci. (Paris) **153**: 1175–1177.

——. 1912. Mode de fixation du calcium par l'*Aspergillus niger.* Compt. Rend. Acad. Sci. (Paris) **154**: 1308–1310.

——. 1917. Le rôle physiologique du calcium chez les végétaux. Rev. Gen. Sci. **28**: 101–108.

Sawine, P. 1916. A propos de la définition de la fertilité des sols au moyen de l'analyse des plantes. Russ. Jour. Landw. **17**: 1–12.

Schimper, A. F. W. 1890. Zur Frage der Assimilation der Mineralsalze durch die grüne Pflanze. Flora **73**: 207–278.

Seissl, J., and Gross, E. 1902. Ueber den Kali- und Phosphorsäuregehalt der Blattaschen verschieden stärkereicher Kartoffelsorten. Zeit. Landw. Versuchsw. Oesterr. **5**: 862–875.

Stahl-Schröder, M. 1904. Kann die Pflanzenanalyse uns Aufschluss über den Gehalt an assimilierbaren Nährstoffen im Boden geben? Jour. Landw. **52**: 31–92, 193–268.

Takeuchi, T. 1908. On the absorption of varying amounts of lime and magnesia by plants. Bull. Coll. Agr. Tokyo **7**: 579–581.

Thacher, R. W. 1913. The chemical composition of wheat. Wash. Agr. Exp. Sta. Bull. **111**: 1–79.

Thacher, R. W., and Arny, A. C. 1917. The effect of different rotation systems and of fertilization on the protein content of oats. Jour. Amer. Soc. Agron. **9**: 344–348.

Tretiakov, S. S. F. 1913. The influence of the mode of cultivation on the chemical composition of the grains of cereals. Trudy Poltav. Selsk. Khoz. Opytn. Stantsii **12**: 28–44. Abstr. in Russ. Jour. Landw. **15**: 83–84.

Truog, E. 1916. The utilization of phosphates by agricultural crops, including a new theory regarding the feeding power of plants. Wis. Agr. Exp. Sta. Res. Bull. **41**: 1–50.

Waynick, D. D. 1918. The chemical composition of the plant as further proof of the close relationship between antagonism and cell permeability. Univ. Cal. Publ. Agr. Sci. **3**: 135–242.

Wiley, H. W. 1901. The influence of environment upon the composition of the sugar beet. U. S. Dept. Agr. Bur. Chem. Bull. **64**: 1–64.

Wilfarth, H., and Wimmer, G. 1903. Die Kennzeichen des Kalimangels an den Blättern der Pflanzen. Zeitschr. Pflanzenkrankh. **13**: 82–87.

Wolff, E. 1871. Aschen Analy, -sen **1**: 1–149; **2**: 1–170.

Wolff, W. 1864. Die Saussereschen Gesetze der Aufsaugung von einfachen Salzlösungen durch die Wurzeln der Pflanzen. Landw. Versuchsst. **6**: 203–230.

——. 1865. Chemische Untersuchungen über das Verhalten von Pflanzen in der Aufnahme von Salzen aus Salzlösungen, welche zwei Salze gelöst enthalten. Landw. Versuchsst. **7**: 193–218.

Harvard Summer School

JULY 5 to AUGUST 13

1921

Courses in Elementary Botany, Morphology, and Plant Anatomy are offered by the Harvard Summer School for 1921. A limited number of research students can be accommodated in Botany S20a. Correspondence in regard to the various courses should be directed to Professor E. C. JEFFREY, 47 Lakeview Avenue, Cambridge, Mass., until the 5th of June. From that date until the opening of the School, inquiries should be addressed to Dr. R. E. TORREY, Department of Botany, Massachusetts Agricultural College, Amherst, Mass. Requests for information may also be sent to the Director of the Harvard Summer School, 19 University Hall, Cambridge, Mass.

UNIVERSITY OF COLORADO

SUMMER QUARTER

Location: Boulder, Colorado, at the mountain front of the Rockies; environment favorable for study, for field work, and for outdoor recreation. Easy access to all of the life zones from plains to alpine heights and glaciers.

Courses in Botany and Zoology: College Botany, Economic Botany, Bacteriology, Botany of Ornamental Plants, Ecology, Local Flora, Forest Botany, College Zoology, Nature Study, Ornithology, Economic Zoology, Cytology, Heredity.

Other Courses in all usual academic subjects.

Summer quarter of two terms: June 13 to July 21 and July 21 to August 27.

Catalogue may be obtained by addressing the Registrar of the University, Boulder, Colorado. For any special information regarding courses in botany and zoology address Professor Francis Ramaley, University of Colorado, Boulder, Colo.

Our Professional Advancement Service

Produces Results

A recent notice brought fourteen replies in four weeks

For terms see elsewhere
in this issue

Address

The Business Manager
American Journal of Botany
Brooklyn, N. Y.

THE ADIRONDACK SUMMER CAMP

OF THE

New York State College of Forestry

AT SYRACUSE UNIVERSITY

June 6 to September 3, 1921

Location: On Cranberry Lake, in the heart of the Adirondack forest.

Field Courses in Plant and Animal Ecology, Mycology and Forest Pathology, Dendrology, Forest Entomology and Silviculture.

Students may register for any or all courses.

Investigators will be welcomed and all facilities of the camp will be placed at their disposal.

General Courses in Botany and Forestry are offered in the Summer Session of Syracuse University, July 5 to August 12, 1921.

For further information, address the **Director of the Summer Session,** Syracuse University, or **Director of the Summer Camp,** N. Y. State College of Forestry, Syracuse, N. Y.

Don't Think We Build But One Kind of Greenhouse

Tiffany sells more than one kind and priced scarf pin. They start in at $5.

That $5 one is just as much a Tiffany pin as the $500 one.

It has the same reliableness.

It is Tiffany through and through.

We build just plain, practical purpose houses, void of all embellishments.

Then we build at least three more, each one to meet a particular need and pocketbook.

There are four ranges of costs.

Still every one is built in the same thorough Hitchings way.

They are Hitchings Standard through and through.

Think of us as high-class builders but not necessarily as high-priced ones.

Tell us your need and we will fit a Hitchings house to it.

Hitchings and Company

Home Office and Factory: ELIZABETH, N. J.

NEW YORK
101 Park Ave.

BOSTON-9
294 Washington St.

Vol. VIII. JUNE, 1921 No. 6

AMERICAN JOURNAL OF BOTANY

OFFICIAL PUBLICATION OF THE
BOTANICAL SOCIETY OF AMERICA

Smithsonian Institution
JUL [illegible] 1921
National Museum

CONTENTS

Specialization and fundamentals in botany . . . . JOSEPH CHARLES ARTHUR 275
Certain aspects of the problem of physiological correlation . . C. M. CHILD 286
Water deficit and the action of vitamines, amino-compounds, and salts on hydration . . . . D. T. MACDOUGAL 296
The eusporangiate ferns and the stelar theory . . . . . . D. H. CAMPBELL 303
The relation of plant pathology to human welfare . . . . . . F. L. STEVENS 315

PUBLISHED
IN COOPERATION WITH THE BOTANICAL SOCIETY OF AMERICA
BY THE
BROOKLYN BOTANIC GARDEN
AT 41 NORTH QUEEN STREET, LANCASTER, PA.

Entered as second-class matter February 21, 1914, at the post office at Lancaster, Pennsylvania, under the act of March 3, 1879

AMERICAN JOURNAL OF BOTANY

DEVOTED TO ALL BRANCHES OF BOTANICAL SCIENCE

ESTABLISHED 1914

EDITED BY A COMMITTEE OF THE
BOTANICAL SOCIETY OF AMERICA

EDITORIAL COMMITTEE

C. E. ALLEN, Editor-in-Chief,
University of Wisconsin

WILLIAM CROCKER,
University of Chicago

L. R. JONES,
University of Wisconsin

C. STUART GAGER, Business Manager
Brooklyn Botanic Garden

ORLAND E. WHITE,
Brooklyn Botanic Garden

R. A. HARPER,
Columbia University

JACOB R. SCHRAMM,
Cornell University

C. L. SHEAR, *Bureau of Plant Industry*
(Representing American Phytopathological Society)

The Journal is published monthly, except during August and September. Subscription price, $6.00 a year. Single copies 75 cents. Back numbers, 75 cents each; $7.00 a volume. Postage will be charged to all foreign countries, except Mexico, Cuba, Porto Rico, Panama Canal Zone, Republic of Panama, Hawaii, Philippine Islands, Guam, Samoan Islands, and Shanghai. Postage to Canada, 20 cents a volume on annual subscriptions; to all other countries in the Postal Union, 40 cents a volume on annual subscriptions.

The pages of the Journal are open to members of the Botanical Society of America, or to candidates approved for membership.

Manuscript offered for publication should be typewritten, and should in all cases be submitted to the Editor-in-Chief.

Papers are limited to 20 pages in length, exclusive of plates, except that additional pages may be arranged for at cost rate to authors (approximately $3.00 a page).

Proofs should be corrected immediately on receipt, and returned to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y.

Illustrations, other than zinc etchings, and tabular matter should, in general, be restricted to approximately ten percent of the number of pages of text. Authors may be charged at cost rates for illustrations and tabular matter in excess of that amount (approximately $6.40 a page for tabular matter; 15 cents per square inch for half-tones).

Separates should be ordered when proof is returned. Ffty copies without cover will be supplied free; cover and additional copies at cost.

Remittances should be made payable to American Journal of Botany. Ten cents must be added to all checks not drawn on New York City banks.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

Correspondence concerning editorial matters should be addressed to Prof. C. E. Allen, University of Wisconsin, Madison, Wisconsin.

Business correspondence, including notice of change of address, and directions concerning reprints, should be addressed to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y., or 41 North Queen Street, Lancaster, Pa.

AMERICAN
JOURNAL OF BOTANY

VOL. VIII JUNE, 1921 No. 6

SPECIALIZATION AND FUNDAMENTALS IN BOTANY[1]

JOSEPH CHARLES ARTHUR

(Received for publication January 10, 1921)

It was my pleasure and good fortune to assist in the launching of the parent society of the present Botanical Society of America, an event which took place somewhat more than a quarter of a century ago. American botany was a lusty youngster among the sciences at that time, but was not generally regarded as capable of doing a man's work in this work-a-day world. It was in good repute within limited circles, but was not consulted when large enterprises were in hand. Even fellow botanists abroad felt no compelling inclination to recognize the work done in America.

Realizing that this condition ought not to continue, steps were taken to organize a society which should exemplify the best thought and endeavor of American botanical activity, and especially should promote higher attainments and a greater amount of original investigation. In order to finance the movement the members were willing to tax themselves with heavy annual dues. The results were increasingly encouraging. After a decade the society united with others into the present more democratic, less burdensome, and more diversified organization, which now stands as the peer of any association of its kind, either at home or abroad.

It was a happy thought to introduce a banquet into the annual program of the society. There may be those who do not see how eating a good meal once a year in the presence of one's associates can aid materially in increasing the amount and quality of scientific knowledge or give a keener zest to the pursuit of discovery. They overlook the subtle relation that exists between bodily good cheer and intellectual elation. Undoubtedly the employment of savory viands to promote fellowship is as old as the habit of eating, and why should not the same agency be carried a step further and made to promote the cause of research? I speak as if it were a new idea. Yet at a date ten times as long ago as the life of this and its parent society the great investigator, Harvey, discover of the circulation of the blood, took this means to increase interest in research. In 1656, a year before his death, he gave his estate of Burmarsh in Kent to the Royal College of Physicians of London. In doing so he stipulated that once a year a general feast should

---

[1] Address of the retiring president of the Botanical Society of America, read at Chicago, December 29, 1920.

[The **Journal** for May (8: 231–274) was issued May 24, 1921.]

be held within the college, and on that day an oration should be delivered exhorting the fellows and members to search and study out the secrets of nature by way of experiment, and also, for the honor of the profession, to continue in mutual love and affection among themselves. Clearly there is illustrious and time-honored precedent for the Botanical Society of America to issue its invitations to "come and eat four grains of rice," as the hospitable dweller in Venice would phrase his most cordial request for your presence at dinner.

I have chosen for my remarks this evening a title so inclusive that, to relieve apprehension regarding any intention to be encyclopedic, I feel it incumbent to state at the outset that it is only a camouflaged sophomoric trick to secure the opportunity for more or less disconnected comments, although on that account, I trust, not less timely or weighty. I propose to speak from the point of view of the investigator and advanced student, rather than from the more usual pedagogic one of the schoolmaster or pupil.

But first of all permit me to revert to Harvey's suggestion that for "the honor of the profession" the members cultivate "mutual love and affection." In the earlier days of botanical organization in America, the kind of organization that first attempted to embrace the length and breadth of the country, the inspiration for which came through the American Association for the Advancement of Science, there was a predominant homogeneity of sentiment and good will with mutual confidence. That was a period not so remote as to be beyond the memory of some of us. In the main that condition still exists. If in some particulars it has been violated, a remedy could be and should be applied.

When making a tour of European universities and experiment stations some thirty or more years ago, I was particularly struck by the reluctance of many botanists in the German institutions to speak openly about their unpublished investigations. There seemed to be a feeling that, should they disclose any part of what they had accomplished, or had in mind to undertake, some colleague might rush into print and deprive them of their honors. It was not the precaution demanded in an older and more densely settled country against the irresponsible and lawless, causing the Germans to put two locks on each door, while in my western home we did not turn the key in the one lock that might happen to be there. It was rather a distrust of one's fellow workers, a state of mind we have learned to associate with a certain type of bureaucracy; and every German professor was at that time a government official. It seemed to me most absurd and uncalled for, quite unbecoming highminded, conscientious, and trustworthy men of science. Since then, some of the same spirit of exclusiveness and distrust, possibly with a tinge of selfishness, has occasionally become manifest in American botanical circles, and it is not surprising to find that it crops out most from government centers. It would be natural to suppose that those who are paid and supported in their scientific work from funds derived impartially from all the people would feel that their first obligation was to the public, at least to the scientific part of the public. But such is the insidious influence of centralized power, or, as G. R. Lyman says, "the zeal of public service," that in some quarters the wealth of opportunity and material are guarded with miserly oversight for the advancement of particular workers and the prestige of the organization. I am not speaking of the individual. There are always a few lacking in a nice sense of propriety and the distinction of *meum* and *tuum*, who must be guarded against. My experience leads me to believe, however, that they are few in botanical circles, both in this country and abroad. What I have in mind is the spirit of exclusiveness, the dog-in-the-manger policy, which should be frowned upon, and so far as possible eradicated from all efficient and truly democratic centers for scientific work. Otherwise, how are we to bear out Harvey's admonition "to continue in mutual love and affection" for "the honor of the profession?" The man of science should be able to appreciate and exemplify what Chaucer means when he says of his knight, that

> he loved chivalry,
> Truth and honor, freedom and courtesy.

At the present day all botanists are specialists. The expansion of the science under many diversified heads, linking it up with other sciences, and the desire to excel in some restricted field, rather than to be content with the level of general information, doubtless explains much of this tendency. With increase in the number of botanical workers has come an increase in the number of organizations bearing distinctive titles. So speedy and intense has been this movement that it has carried some of the younger members of the profession quite off their feet, and in the rarefied atmosphere of their new environment they no longer see the solid earth from which the maintenance of their strength must come. Oh, no! they say, I am not a botanist, I am a pathologist, an ecologist, a geneticist, or what not. Let us hope that the allurements of the silver-lined clouds of science will not keep them from eventually considering the mists that bedew the earth and renew its verdure.

Probably the most fundamental thing that the specialist is likely to neglect is an intimate acquaintance with the plants with which he deals. When the distinctive instrument of the botanist's labors was the vasculum, now rarely seen, and to be credited with a knowledge of elementary botany required one to pass in fifty named and mounted specimens, only the indolent missed a suitable basis for botanical advancement. When the microscope became the botanist's chief instrument, the foundations began to be neglected in the construction of the multivaried details of the superstructure. With the advent of other instruments of research, the microtome, the auxanometer, the atmometer, etc., attention was directed more and more away from the individual plant as an interesting inhabitant of the organic world. The study of structure, physiology, or behavior has often proceeded with inadequate knowledge of the position that the particular plant under observation holds in relation to other plants, its resemblances and differences as compared with its kin, near and remote. When Gray's *Lessons in Botany* was superseded by the general textbook that began with slime molds and ended with sunflowers, there did not seem to be time to make the acquaintance of particular plants, and when a little from each topic pertaining to the varied structure and action of plants from the cytology of the cell to the sensitiveness of a tendril had to be interpolated, the plant as an individual member of an evolutionary group was overshadowed. The great English botanist, Sir Joseph Hooker, once wrote to Dr. Asa Gray:

> I content myself with a casual grin at young men calling themselves botanists, who know nothing of plants but the "innards" of a score or so. The pendulum will swing round, or rather back, one day.

It is already on the way; let us hasten the movement.

The botanist's realm is the vegetable kingdom. As the man of the world is able to assign each person he meets to a particular race, country, or section, with more or less accuracy, to have some individual acquaintance with a few here and there, but knows only those within a limited circle sufficiently well to call them by name and to be familiar with some of the facts of their history, so the botanist should have a general knowledge of plants of all countries sufficient to enable him to place most of those coming to his attention within certain orders or families, to know a few by name, and with those he meets frequently, especially the flowering plants, to have the same familiar acquaintance of name, characteristics, and behavior, which he prizes for his speaking friends. In the realm of plants the botanist has a distinct advantage over the man of the world, for he has manuals which enable him to ascertain the name of the plant he wishes to know, and to be unfamiliar with such manuals is to write oneself down inadequately equipped for his duties. My attention has been most frequently called, perhaps, to the shortcomings of the cytologist, who essays to throw light on the relationship of parasitic fungi by a discussion of his observations without taking full precaution to make sure of the exact identity of the material he has used, or of the kinship of the forms he has selected for comparison. In this way laborious and extensive studies may fail to exert due influence, and may have their chief value confined to a record of the particular observations. But no class of investigators need be credited with an unwarranted share of haphazard interest in the exact identity of the plants handled. I fancy the bacteriologist and the paleobotanist have the best reasons for being uncertain. There is a joke, with which you may be familiar, that, when puzzled about the affinities of a plant, "fossilize it and send it to a paleobotanist, and he will give you the genus and species at once." The students of microfungi are not to be outdone in this particular. Among parasitic forms the relation of the fungus to the host is very intimate, and the identity of the one often involves that of the other. Examples are numerous where the name of the host has been adopted for the fungus growing upon it, only to learn later that due care had not been exercised by the original collector and that the host was not what it purported to be, the name thus becoming a misnomer. In general, probably, a considerable percentage of the inaccuracy and misinterpretation in various fields of botanical science is traceable to a lack of intimate acquaintance with plants as living objects having distinctive names and varied relationships.

While systematic botany may never again have the place of honor in the curriculum that it held in the post-Linnaean days when Jean Jacques Rousseau wrote his delightful letters on the elements of botany, and coming down to the days of our own beloved Asa Gray, yet no man who essays to explore the domain of plant knowledge, whether as student, investigator, or philosopher, can afford to be without an understanding of its main tenets, based upon spirited contact with the plants of the field and upon ability to localize and identify individuals engaging his attention.

As somewhat of an accompaniment to these thoughts, but quite as an independent theme, I bespeak consideration to the matter of names. So fully have the taxonomists been shoved aside in recent years that devotion to the task of disentangling, rectifying, and correctly assorting the mass of names in any group of plants appears to many botanists as a work of supererogation, largely futile, and almost finical. They are reputed to be meddlers, with a penchant for displacing well known names by unfamiliar ones, and possessed of an insatiable and egotistical desire to see their own names appended to as many Latin designations as unlimited juggling may seem to give warrant. Moreover, there is apparently a feeling that there are names enough in use, at least enough for all except a few rare species in out-of-the-way regions yet to be brought to light by explorers; and that if the nomenclaturists would let them alone we should not be obliged to learn a new set of names, and to puzzle over their identity with the old ones, every time a fresh work on botany comes from the press.

There is plenty of justification for irritation over the nomenclature situation. All will agree that each plant should have its fixed name independent of any particular botanist's certification. But we are far from that goal at present. Why? Is it an impossible goal, or do we needlessly muddle the situation and retard progress?

Of course every active systematic botanist knows how false is the prevalent idea that most plants have been sufficiently studied to make their identification as species no longer uncertain. Let it be remembered how few years ago it has been since we became aware that the plantains and dandelions in our lawns were not each one species, as the botanical manuals stated, but that each comprised two species and well defined. In my garden I have grown for a number of years a delectable small fruit, that I have seen listed horticulturally as Garden Huckleberry, and that evidently belongs to the great genus Solanum or one of its segregates, but I have been unable to find it described in any botanical treatise at my command. There may be, and doubtless are, other plants in our front yards and vegetable gardens whose naming is uncertain for lack of sufficient study, and how much more so must be the case of plants in forests, fields, and mountains, and in the botanically unexplored regions of our own and other countries. What is true of the larger flowering plants is even more applicable to the far greater numbers of the less conspicuous lower orders of plants.

The introduction of any number of new names, when discriminately applied to really new species, is not a source of embarrassment, but an aid to better understanding. The trouble arises when two botanists in different parts of the world independently give different names to the same plant, or when a name is applied to a species supposed to be new but afterward found to have been named, or when some one ascertains that a name has been badly chosen, is inapplicable, or of faulty construction, or when the demands of classification seem to require the transfer of a name from one genus to another. In such or similar cases, which are exceedingly numerous, the choice of rival names is still largely a matter of personal preference, although from the days of De Candolle attempts have been made to formulate guiding rules, which have been of more or less service, but never generally accepted. It is the opinion of Mr. C. G. Lloyd of Cincinnati, whose trenchant pen has scored many present-day mycological nomenclaturists for their pedantic ways, that the value of a name should be derived from "historical truth and general use." He believes that "if mycological writers in general would rely on these principles alone in the selection of names, it would only be a short time until we should be in practical accord." The principles seem simple, and if they would serve to secure acceptable unity for mycological names, they would doubtless serve as well for all other plant names. Certainly the great desideratum for names is their general acceptance, so that the same name always applies to the same plant in the writings of all authors. More than a century ago, when the controversy was raging in this country over the comparative merits of Jussieu's natural system and the artificial system of Linnaeus, Thomas Jefferson, "one of the six greatest men in the history of the public life of the United States," as a recent historian has stated, a broad-minded statesman and a man of high scientific attainments, contended that in this connection no matter was " so important a consideration as that of uniting all nations under one language in natural history." The committee on nomenclature appointed by this society is endeavoring to aid in such a movement. As no rules to govern the names of plants can be made mandatory, their general acceptance must necessarily depend upon their appeal to precision and serviceableness, as well as upon the provision they make for authentication in doubtful cases.

As already indicated, the regulations for selecting and validating the correct name of a plant have been slow in taking shape. It has long been recognized, more and more strongly of late, that the name first given to a particular species of plant must be considered its proper and legal name. The difficulty has been to secure agreement upon the particular name to be considered as having precedence. The difficulty is somewhat the same as the courts of justice have in proving that the name on the docket properly belongs to the person before the bar. The latest move among nomenclaturists is to follow the methods of the law courts, wholly abandoning the attempt to prove that the name is correctly used and being content with making sure of the identity of the person in question, or, in botany, establishing the identity of the particular specimen of plant which was in hand when a name was published. This is known as the type-basis method, and promises to bring definiteness and exactness where before was the uncertainty of individual interpretation. Nature has not provided us with species and genera, but only with individuals having greater or less resemblances. As botanists we find it convenient to treat individuals possessing a certain amount of resemblance as species, and these species we group into genera. The size and variability of the units we call species and genera will depend in each case upon the taxonomic views held at the time, but the name, according to the type-basis method, must always find its application in accord with the characteristics possessed by the original specimen upon which it was founded.

Having now said something about the desirability of knowing plants at first hand, and about the application of their names, permit me to say a few words about the names themselves. Since the days of Linnaeus, names of plants have been binomial, with tendencies now and then to become trinomial, quadrinomial, or even multinomial, but never monomial. Evolution of the onomatology of plants has many parallelisms to that of persons. In the early days, that is, before the middle of the eighteenth century for plants, and before the tenth century for persons, names either of plants or of persons might consist of a single word, or on the other hand might be of indeterminate length. For persons there was a gradual evolution into a surname and given name, while for plants a far more rapid change brought about the corresponding generic and specific name. The names of persons are not established by law, but by usage. The first name applied to an individual, however obtained, is almost invariably accepted in after years, and yet there is no law in this country or in England, and certainly not elsewhere, against changing a name; nevertheless, certain states have provided a process by which a change may receive legal sanction, if such is desired. The case of plants is almost identical, except the last provision for validating a name. But the movement is well under way to provide fixed rules to serve as a guide in the bestowal of plant names, to indicate the correct names previously given, and to secure their maintenance, which should eliminate much of the present confusion; even the provision for validating a name by a fully authorized tribunal, when a change is desirable, is being considered.

In other ways the usages regarding personal names and plant names show a similarity in evolution. In the earlier days personal names usually denoted some quality or distinction in the individual, fanciful or real; as Clovis, glorious warrior; Mathilda, mighty amazon; Adolf, the noble wolf; Cicero, the vetch-grower. When surnames came into use they were at first selected in much the same way, as Rich, Noble, Black, Brown, Archer, Goldsmith. But after a time the multiplicity of names, and the necessity of continuing their use when no longer applicable, led to the present usage of disregarding the qualifying significance in either surnames or given names, and to select them for euphony, or family association, or fanciful reasons. The course with botanical names has been much the same, but the evolution has not gone as far, doubtless because of the shorter period of time involved. For a while it was thought necessary to give descriptive or informative names to plants, and when they proved inappropriate to change them. But the practice has largely fallen into disuse in late years. *Plantago major* is a much smaller plant than the similar and more common plantain that grows with it everywhere in this country east of the Rocky Mountains, yet the name has not been changed. Some taxonomists, however, who deal with the lower orders of plants, especially the fungi, are still in the dark ages with their nomenclatural practice. A rust called *Puccinia Distichlidis* has been renamed, because it was found that the grass on which it occurs is not Distichlis, but only looks like Distichlis. In another group of fungi a prominent writer stated not long ago that

> We have heretofore used *Cyathus Poeppigii* as the name for this species, but in the future we shall adopt the name originally applied to it by Poeppig. We do not do this on any ground of priority, but because *Cyathus Poeppigii* is a heathenish kind of name that ought to be suppressed.

Within the last month or so a transatlantic colleague has written regarding a species of Helminthosporium:

> May I point out the course we have adopted with regard to the spelling of the specific name of the barley stripe fungus? We now invariably use *graminum*, the genitive plural of the substantive, believing this to be more correct than the adjectival form *gramineum*.

One might cite many instances to show that, although the latest rules of nomenclature do not sanction changes like these, we are yet slow in arriving at the stage at which the name of a plant, like that of a person, is generally considered as an appellation wholly for identification, and whatever of descriptive or adjunct information it may convey must be considered entirely incidental or historical.

Curiously, we speak of the name of a plant or person as if it were a simple designation, like a number. Yet it is a compound of two very unequal parts. When we say that the correct name of a plant is the one first applied to it, we mean the specific name only, the one corresponding to a person's baptismal name, and it is toward this part of the name that most of the rules on nomenclature are directed. The specific name may be transferred from one genus to another as many times as seems desirable, in order to express its relationship, just as a woman's surname changes upon remarrying, or a man may take another surname to meet the requirement of a bequest; but the identity of the plant as of the person is inherent in the specific or baptismal part of the name, although standing by itself it would mean little. Thus the common field thistle, which we usually call the Canada thistle, was named by Linnaeus *Serratula arvensis.* At intervals of a few years it was successively transferred by different authors of the old time to the genera Cirsium, Carduus, and Cnicus, but at present is most generally listed as *Cirsium arvense*, I believe. Again, Linnaeus called the common dandelion, that makes our lawns glorious with golden bloom in spring and later turns them into a ragged waste, *Leontodon Taraxacum*, the specific name being adopted from an old-time classical name. Later this genus was divided, and the dandelion dropped into the new genus Taraxacum, it being called *Taraxacum densleonis*, which had the same meaning as did the first name. But it is now contended that the earliest specific name is the rightful one, irrespective of meaning, and in consequence the dandelion should be called *Taraxacum Taraxacum*, which strangely enough is a combination that is strenuously objected to by a large number of botanists. Why should this and the like combinations, *Sassafras Sassafras*, *Abutilon Abutilon*, etc., be any worse names for plants than William Williams and Smith de Smith for persons? Until we bring ourselves to look upon plant names as simply names, not qualifying terms, our science will be handicapped by the impedimenta of prejudices whose rightful place is in the musty volumes of the antiquary.

Now a word about those appendages of every Latin botanical name, which C. G. Lloyd calls the personal advertisements. In the present unsettled state of nomenclature they are as necessary for keeping names from going astray as the tail is necessary to guide a tadpole. When plants become better known, and names are more consistently applied, the caudal appendages will be dropped as burdensome and useless. To get us out of the tadpole stage in nomenclature, however, will assuredly take united effort and willingness to forego prejudices. Every botanist should be interested in hastening the day. Whatever one's specialty, he must use the Latin names of plants. Exact names, uniformly applied, are a fundamental requirement of the science, and it is in the interest of every botanist, as well as of horticulturists, agronomists, and all other users of plant names, to hold a favorable attitude toward attempts to secure this end.

At this point I am reminded that the most prominent feature of the present movement in botanical thought is organization and coöperation.

We are clearly entering a new era for scientific labors. Research has become the watchword of the hour and is to be encouraged more than ever before. In course of time it may even be acceptable and intelligent to officialdom for one to give his occupation as *investigator* and not to be obliged, as at present, to masquerade as a *teacher*, even when he does no teaching and possibly may not be connected with a teaching institution. It is now commendable not only to encourage the spirit of research, but to assist in providing a general atmosphere favorable to its development. Naturally, as in other movements that become popular, there is sometimes more talk about the value and desirability of research, than actual accomplishment, or even hearty direct assistance in providing time and means for its prosecution. Nevertheless, we are likely to see the number of research centers, both great and small, much increased, and the ranks of those who essay the task of adding to available knowledge immensely augmented.

In the re-awakening and re-orientation of the research spirit it should be possible to preserve and advance that fine democratic quality which recognizes, as we were reminded by one of our number two years ago at the Baltimore meeting, that "botany is a world science and that its advance can not be accelerated through the usual operation of institutional or individual rivalries." There must be the fullest and freest coöperation between institutions, and quite as much between individuals, both as members of organizations and as independent workers. Harvey's exhortation to mutual consideration should find practical fulfillment, both for the good name of the profession and for the efficiency of its labors.

While I am pleading for individual freedom and encouragement against the encroachments of the machinery of organization, I do not undervalue the great service and importance of associations, both those of voluntary combinations of individuals and those centering about institutions. I subscribe most heartily to the views of Mr. Frederick W. Taylor, who had in mind especially the research conditions in the commercial world, but whose words are equally applicable to things botanical, when he said:

> The time is fast going by for the great personal achievement of any one man standing alone; and the time is coming when all great things will be done by that type of coöperation in which each man performs the function for which he is best suited, yet preserves his own individuality and initiative and is supreme in his particular function, while controlled by and working harmoniously with many other men.

These words breathe the true democratic spirit of personal freedom as against the bolshevistic absorption of the individual in the organization.

In the movement for greater accomplishment by means of organization the class of problems which are uppermost for consideration are the economic ones, or those which can be justified by a direct popular or commercial demand. These are the ones for which money can be most readily obtained, and in which the largest number of persons can be interested. These are the ones chiefly supported by the general government, because they are nearest to the interests of the taxpayer to whom the government must appeal for funds. They are most likely to receive attention from state institutions whose success depends upon heeding the popular demand. Even privately endowed educational institutions and detached research institutions can not help but be influenced by this tendency. Such problems have almost monopolized the word *specialize.* Thus Dr. Lyman says:

> The agricultural institutions have specialized too strictly and have laid too little stress on the fundamentals of botany.

With the natural instinct to be interested in the under dog, my closing words shall be a plea for greater attention to the fundamentals in making provisions for organized support. The solution of problems falling in this class furnishes the tools for the specialist. Some phases of taxonomy, of which I have already spoken, might be used as an example. The consistent, effective onward march of botany calls for careful balance between the attention given to specialization and that given to fundamentals.

CERTAIN ASPECTS OF THE PROBLEM OF PHYSIOLOGICAL CORRELATION[1]

C. M. Child

(Received for publication January 14, 1921)

The existence in the growth and development of axiate plants of a relation of dominance and subordination, of control and being controlled, has long been recognized. This relation is very evidently associated in some way with at least certain fundamental physiological activities of plant protoplasm and apparently particularly those which have to do with growth. The active vegetative tips are the chief regions of dominance, but other growing regions may exercise a similar dominance to a greater or less degree. That this relation is a real physiological relation and dependent on the dynamic activity of the dominant part has been demonstrated repeatedly by experiments in which the dominance is abolished by inhibition of the fundamental metabolism and growth of the dominant region, but reappears when the inhibiting factor is removed and the dominant region returns to or approaches its original condition.

My work on so-called physiological polarity, correlation, and integration in animals has shown very clearly that in axiate animals as well as in plants a relation of dominance and subordination exists, not only as regards the functional activities of the fully developed individual, but also in growth and development. The evidence indicates that the functional relations of later stages as expressed in the nervous system and in the chemical interrelation of parts are the consequence and outgrowth of a more general and primitive relation which exists before the nervous system appears and before the various parts differentiate. Since this relation in its more general form as it appears in the simpler animals and the earlier stages of development seems to be very similar to the relation in plants, I have very naturally been much interested in attempting by means of work along various lines with plants to discover whether, or to what extent, such similarity exists. It is because of some of this work on plants that I have been asked to take part in this program. My objection that the work cannot properly be regarded as biophysics any more than biochemics, and that it has not yet attained the exact quantitative character and formulation which would warrant its inclusion in either of these special fields of physiology, was overruled by those in charge of the program, so that responsibility rests upon them.

---

[1] Invitation paper read before the Physiological Section of the Botanical Society of America, in the symposium on biophysics, at Chicago, December 28, 1920.

A very brief survey of certain lines of work is necessary by way of introduction to the experiments of which I wish particularly to speak. Study of several hundred species, including animals from all the chief groups and many algae and some other plants, have shown that physiological polarity and symmetry in their simplest terms consist of gradients in physiological condition and activity of the protoplasm or cells composing the organism. These gradients have been called axial, metabolic, or physiological gradients. That they have to do with the fundamental physiological condition of the protoplasm is clearly shown by the many different lines of evidence which demonstrate their existence. They appear as gradients in susceptibility to certain toxic ranges of concentration or intensity of external agents, *e.g.*, cyanides, heavy metal salts, anesthetics, acids, alkalies, other neutral salts, CO_2, various dyes, extremes of temperature, and the negative condition, lack of oxygen. Within certain limits of concentration or intensity these susceptibility gradients are non-specific, *i.e.*, essentially identical in their larger features with all external agents tested, at least in the simpler animals and plants and in the earlier stages of development of higher forms. It has been shown that these differences in susceptibility are indicators of differences in rate of fundamental metabolism, particularly oxidation. The physiological gradients can be demonstrated as gradients in the rate of penetration of non-toxic or only slightly toxic vital dyes. Again, in dilute solutions of the oxidizing agent $KMnO_4$ they appear as gradients in rate and amount of reduction of the salt. In certain cases the indophenol reaction has been used, and a gradient in the rate of appearance of the indophenol suggests a gradient in oxidizing enzymes. A gradient in electric potential is a characteristic feature of the physiological gradient in all forms thus far examined, though in some plants the electrical situation is apparently complicated by the occurrence of reactions which give rise to opposite potential differences, *viz.*, the oxidations and photosynthesis. And finally, in animals in which it has been found possible to determine the oxygen consumption and CO_2 production of different regions along the axis, differences corresponding to those indicated by other methods have been found. It has not yet been possible to apply all these methods to each species examined. These physiological gradients also very commonly appear in differences in structure of the protoplasm along the axis, as in many plant and animal embryos, and they are definitely related to differences in rate of development and differentiation.

It has also been possible to show that the localization and differentiation of organs and parts occur in a definite relation to the physiological gradient, in fact are determined by it. The most active region as finally determined, *i.e.*, the region of highest susceptibility, of greatest permeability, of greatest reducing capacity, of highest external electro-negativity, and of highest rate of respiration in the polar axial gradient, becomes the apical end of the axis, or in animals the head, and the other organs develop at different levels of the gradient. The question how the primarily quantitative differences in such a gradient can give rise to the qualitative differences characteristic of differentiation of cells presents no fundamental difficulties. Differences in the relation between available nutritive substance and the rate of oxidation at different levels of a gradient undoubtedly determine the appearance of certain substances in the cells at one level and their absence at another. Differences in concentration of certain substances at different levels may also determine the formation of different products, and various other factors in the complex protoplasmic system doubtless play a part in determining the origin of qualitative differences from the quantitative differences of the gradient. But whatever the local factors involved in each particular case, the physiological gradient constitutes the primary factor in determining localization and differentiation of parts along an axis.

The important point for present purposes is that in such a gradient a relation of dominance and subordination exists, the high end, the most active region, of the gradient being the dominant region and determining to a greater or less extent conditions at other levels within a certain distance, which differs with the stage of development, the condition and differentiation of the protoplasm, and the degree of activity of the dominant region. Since this dominance is effective only within a certain range or distance, the possibility of physiological isolation exists, that is, either in consequence of increase in length of the organism, of decrease in activity of the dominant region, or through a blocking in some way of the passage of the controlling influence, certain parts may become isolated from the action of the dominant region, even though still in physical continuity with it. In the simpler animals and plants such physiological isolation results, like physical isolation, in dedifferentiation and development of new axes, or parts already present but previously inhibited, such as latent buds in plants, become active and develop.

The question of the nature and origin of physiological gradients is obviously of fundamental importance. The gradients, so far as can be determined, represent primarily quantitative rather than qualitative differences, and if this is true, as all the evidence indicates, the relation of dominance and subordination cannot be fundamentally a matter of chemical or transportative correlation, that is, of mass transportation and action upon one part of specific substances or hormones produced in another. In order that such correlation may exist the parts concerned must already be qualitatively different, and the evidence indicates that dominance and subordination exist in the absence of such differences. Unquestionably chemical correlation is of great importance as soon as qualitative differentiation begins, but it cannot be the primary factor in correlation and in determining such differentiation in the organism.

The only other possibility appears to be the transmission of dynamic change of some sort, that is, of excitation. We must therefore inquire whether the physiological gradient shows any similarity to an excitation gradient. All living protoplasm is excitable and to some degree capable of transmitting excitation. Excitation in its most primitive form appears to be an acceleration in the rate of living, particularly as regards the energy-liberating aspects of life. Where specialized conducting paths are not present transmission of excitation occurs with a decrement, that is, the transmitted change becomes weaker and finally disappears at a greater or less distance from the point of origin. Such a process of excitation and transmission gives rise to an excitation-transmission gradient. We usually think of such gradients as temporary or reversible, but the physiological gradients show all the characteristics of excitation gradients which have become more or less permanent.

Moreover, it has been shown experimentally that these gradients can be produced in cells or cell masses by subjecting them to a quantitative differential in the action of external factors. For example, new gradients and so new polarities can be determined experimentally in the simpler animals by a sufficient difference in oxygen supply, by an electric differential, perhaps in some forms by a light differential, and probably also by various other differentials. A differential inhibition may have the same effect as a differential excitation or acceleration. Turning to the plants, the polarity of the Fucus egg, which is primarily a gradient, is determined by the differential action of light, and the polarity of the Equisetum spore has a similar origin. The relation of dorsiventrality and symmetry to light in the plants is a familiar fact. In order to establish a gradient sufficiently persistent to serve as a physiological axis, the differential action of the external factor must persist for a certain length of time dependent on the nature and intensity of the factor and the character of the protoplasm. The fixation of such a gradient in protoplasm must depend upon the occurrence at the different levels of changes which are more or less irreversible under the existing conditions and which differ in degree according to level. Once established in a cell or cell mass, such a gradient may persist through division or other reproductive processes and become the basis of the axis of the new individual or individuals. In other cases the original gradient may disappear in reproduction and a new gradient arise. In many eggs, both animal and plant, the gradient is apparently determined by a differential in relation to the parent body, such, for example, as the difference in conditions at the attached and the free end of the egg; but in some eggs it may perhaps persist from earlier cell generations, while in others, as in Fucus, it is determined by a factor external to the organism.

We come now to the question of the nature of the dominance or control, and this involves the question of the nature of transmission. Many hypotheses have been advanced concerning the process of transmission, and most of them connect it in one way or another with the electric changes which are a characteristic feature of excitation. On the basis of extensive experimental investigation R. S. Lillie has developed an electro-chemical conception of excitation and transmission which seems to interpret and account for the various phenomena more satisfactorily than others previously advanced. The unexcited surface layer of the cell behaves as if more permeable to positive than to negative, or to certain negative, ions, and is therefore electrically polarized. Excitation increases its permeability to the negative ions and depolarization results, with an increase in electronegativity of the external surface. In this change a chemical reaction, an oxidation, is involved, whether as the primary or as a secondary factor is not at present known. The electric current arising at any point of excitation becomes the factor determining depolarization and excitation at all points within a certain distance, beyond which it is too weak to be effective, and each new region of excitation becomes the source of current which may, if strong enough, excite further points. At the same time the current tends to restore the polarization at the point of original excitation and so to reverse the excitation process at that point. By means of this current, then, according to Lillie, transmission occurs. With simple inorganic models he has been able to demonstrate the occurrence of transmission in this way, both with and without decrement and at different speeds, as well as the development of fixed gradients. The speed of transmission has no relation to the speed of electrical transmission, but depends on the velocity of the changes at each point of excitation which give rise to the current. The development of fixed gradients occurs when conditions determine the persistence of the region of high potential which in turn determines a potential gradient extending over a greater or less distance and so a gradient in the conditions determined by the electric current.

Whether this theory of excitation is in all respects correct or not, it enables us to see how a region of excitation in undifferentiated protoplasm may determine the origin of a physiological gradient. The facts indicate that these gradients do arise in this way, and if we admit this, it follows that the primary factor in dominance and subordination is transmission. The establishment of a region of high activity must affect adjoining regions within a certain distance as a region of excitation affects them, and it is difficult to believe that the electric potential characteristic of such a region is not a factor and probably the primary factor in such a relation. If the degree or intensity of excitation at each level is in any degree proportional to the strength of current, a gradient must result, and if the conditions determining the gradient persist for a certain length of time, changes in the protoplasm at the different levels may determine the more or less permanent fixation of the gradient. In most protoplasms this relation between stimulus and excitation does exist, and a gradient results from local excitation. In the nerve fibers of the higher animals, however, the excitation process is specialized so that any stimulus above the threshold gives rise to maximal excitation and there is therefore theoretically no decrement in transmission.

The relation of dominance and subordination does not necessarily persist in its primitive form throughout life. In animals, for example, the development of the nervous system, with highly specialized paths capable of transmitting excitation so much greater distances than embryonic protoplasm, makes possible a much more complete and extensive dominance, which nevertheless is built up on the basis of the primitive relation. On the other hand, the qualitative differentiation of different organs affords a basis for complex chemical or transportative correlation. In plants, buds which are inhibited for a time may sooner or later become incapable of development, even when physiologically isolated, either because they have not been able to develop channels for the passage to them of water and nutrition, or because of changes in the cells in consequence of the action of the dominant region upon them. On the other hand, even in plants the conductivity of certain tissues may increase with differentiation and dominance be possible over greater distances than at first. In short, the primitive transmissive relation may develop and attain greater importance in certain types of relation, or it may be supplemented or even replaced by chemical correlation, or, finally, with advance in differentiation of parts the correlative factors may be chiefly nutritive. In any case the situation in the plant remains much simpler than in the higher animals, in which both the transmissive and the transportative relations are extremely complex.

My work on plants, in which Dr. A. W. Bellamy assisted me, was undertaken with the hope of being able to throw some light on the question of the nature of dominance and subordination as it exists in the growing plant. Thus far I have merely succeeded in blocking by means of low temperature the correlative factor on its way without interfering to any marked degree with the flow of fluids in the plant. As I have pointed out elsewhere, the results favor the view that correlation is accomplished by a transmission rather than by mass transportation of special substances, but much remains to be done before positive conclusions are permissible.

My experiments thus far have been chiefly with three plants, *Bryophyllum calycinum*, *Phaseolus multiflorus*, and *Saxifraga sarmentosa*. The method of experiment and the results obtained with Bryophyllum have already appeared in the *Botanical Gazette*. As regards method, it may be said here that the low temperature is applied by surrounding the zone to be cooled with a coil or loop of small block-tin piping which can readily be bent and adjusted and through which water of controlled temperature flows. The region to be cooled is first wrapped in tinfoil and direct contact with the piping is avoided, the space between the coil and the plant being loosely packed with slightly moistened absorbent cotton, and the whole region of the plant and the coil after adjustment well wrapped in order to reduce temperature change from external sources to a minimum. All cases in which visible external injury due to pressure or to too low temperature occurs are discarded. Temperatures ranging from 3° to 8° C. are used according to the plant and the particular object in view. In the bean seedling cell turgor is somewhat reduced in the cooled region after some days, particularly in the lower temperatures. On removal of the coil, however, normal turgor is reëstablished within a few hours, and if the temperature is raised gradually at the end of the experiment the turgor is normal when the wrapping is removed.

In the experiments on Bryophyllum[2] the low temperature is applied to a zone of the petiole 2–3 cm. long, and the leaf is immersed in water so far as its position on the plant will permit. The opposite leaf of the same node and usually leaves of other nodes above and below are also immersed. In the experimental leaf all or nearly all the immersed buds develop, in the opposite leaf there is as much or almost as much development in most cases, and more or less development occurs in leaves of nodes above and below the experimental node. Controls with leaves immersed but without the low temperature often show development of a bud here and there, but the effect of the low temperature is clear and unmistakable in the experimental leaf, in the opposite leaf, and to a less extent in leaves of neighboring nodes.

The case of the scarlet runner bean is of greater interest than that of Bryophyllum, for here the low temperature is applied to the main stem of the seedling, and all water and nutrition passing to parts above must pass this zone. If the low temperature interferes appreciably with the flow of fluids, this should be evident in the retardation of growth above the zone, or in extreme cases in wilting. There is in some cases slight retardation of growth of the tip, particularly when the low temperature is applied to the upper part of an internode of the young seedling in which elongation is still going on and the vascular bundles are still developing. Except when the temperature is very low, however, this retardation is only temporary and the rate of growth of the tip increases even before the low temperature is removed, and in no case is the effect on the tip sufficient to decrease its dominance to such an extent that axillary buds above the low temperature zone develop. Morevoer, that this retardation has nothing to do with isolation of buds below the cooled zone is shown by the fact that a temperature of 5°–6° C., applied to the upper end of an internode, produces at first marked retardation of growth of the tip but no growth of buds below, while the same temperature applied near the lower end of the internode produces no appreciable retardation of the tip, but the buds in the axil below develop. In general, the farther away from the axils to be isolated the low temperature is applied, the less effective it is in producing growth of the buds and *vice versa.* These facts suggest that the inhibiting factor, if it passes at all through a cooled zone, undergoes a gradual return or approach to its original effectiveness in its further course, so that when the cooled zone is farther away from the buds to be isolated it is less effective. Moreover, with temperatures which are not too low, a long cooled zone is more effective as a block than a short one, but with sufficiently low temperatures the short zone is effective. In these respects the correlative factor apparently behaves to some extent like the nerve impulse.

---

[2] In the original presentation of these and other experimental data lantern slides were used.

Growth of the buds isolated from the tip by low temperature is usually evident within one to two days. If the temperature is near the upper limit of effectiveness, the growth of the buds below the zone usually ceases after a few days in spite of the presence of the cooled zone. This is undoubtedly due to the occurrence of some degree of acclimation in the cooled zone with consequently more effective passage of the block by the inhibiting factor. If the buds are allowed to grow for ten days or more before the removal of the low temperature, they usually continue to grow more or less rapidly afterward, and in this way plants with three or more stems can be produced. Earlier removal of the low temperature usually results in renewed inhibition.

In my experiments with the saxifrage, the low temperature is applied to a zone of a runner which has not attained its full length, with the result that the runner soon ceases to elongate and begins to develop a new plant at the tip, even when suspended in air. Here also water and salts reach the runner tip only by passing the cooled zone, and the rapid development of the new plant shows that this flow is not seriously affected. According to Loeb's hypothesis, it seems that substances inhibiting the development of the new plant at the runner-tip must be transported by this current, but as a matter of fact the low temperature isolates the tip without stopping the current.

Whatever the nature of the correlative factor may prove to be, these experiments, particularly those on the bean seedling, seem to me to offer difficulties to the hypothesis that this factor consists of an inhibiting substance or substances transported in mass through the plant. Since the correlative factor can be blocked by a zone of low temperature, we must assume, if it consists of a substance or substances, either that it is transported through the living protoplasm and that its passage is dependent upon the physiological condition of the cells, or that it is of such a nature that it is precipitated out of, or otherwise removed from, the fluids of the plant as they pass the cooled zone.

On the basis of the first assumption, we should expect a substance which inhibits the growth of vegetative tips to inhibit the growth of the cells through which it passes. Below the chief growing tip, for example, such a substance must pass through the region of most active growth in the axis, but it does not inhibit this region. In fact, if such a substance passes through the living cells of the plant, most complex and remarkable relations of immunity and susceptibility must exist. Each growing tip, for example, or any other part producing such a substance, must be immune to the substance which it produces, but other growing tips are susceptible to it.

The alternative assumption, that the substance is transported in the fluids of the plant and removed in some way from them in the cooled zone, does not serve any better than the first for the interpretation of certain experimental results. In fact, the hypothesis of inhibiting substances and their transportation, in whatever form we state it, does not account for the fact that within certain limits of temperature the effectiveness of a cooled zone of certain length in the stem of the bean seedling decreases with increasing distance from the buds to be isolated, even though the more distant zone may be more effective in inhibiting the growth of the chief tip. In other words, a cooled zone which has a marked inhibiting effect upon the movement of water and salts in the stem is not necessarily effective in isolating buds below, while a zone which has no appreciable inhibiting affect on regions above it may be effective in isolating buds below.

Assumptions concerning the transportation of nutritive substances are not, I believe, any more satisfactory than the hypothesis of inhibiting substances in aiding us to account for the facts of physiological correlation in the plant. As already noted, physiological isolation of buds below a cooled zone may be brought about without retarding the movement of water and salts to any marked degree. In the case of Bryophyllum, the leaf itself is able to produce starch, and in the bean seedling the reserves of the cotyledons are available for the buds in the axils of the cotyledons and those produced by the first pair of leaves are available for the buds of the second node. In the saxifrage runner the cooled zone may retard to some extent the passage of nutrition to the runner tip, but the results with the other species show clearly enough that this is not the primary factor in the physiological isolation of parts in plants. In the case of the Bryophyllum leaf with cooled zone about the petiole, the passage of water and salts to the leaf may be somewhat retarded, but the leaf contains plenty of starch. In the bean seedling the passage of water and salts to the buds to be isolated is not interfered with, since the cooled zone is above them, and here also plenty of carbohydrate is available. In both these cases, as well as in the saxifrage runner, physiological isolation and growth of buds occur. Again, if we cut off the stem of the bean seedling below the first foliage leaves and remove the cotyledons, the buds in the axils of the cotyledons, which are now the only buds on the plant, will develop, although in the absence of the more apical parts of the plants the movement of water and salts must be greatly decreased and the removal of leaves and cotyledons must have decreased the amount of carbohydrate available. In short, the result as regards development of the buds is essentially the same under experimental conditions which must determine very different internal conditions as regards the movement of nutritive substances and the amount available in a particular region.

Finally it may be noted that in the bean seedling the rate of reaction of the buds of the different axils to physiological isolation by a cooled zone differs according to the level of the plant. With a cooled zone of given length and given temperature at a given distance above the node concerned, the buds of a more apical node begin to grow earlier and grow more rapidly than those of a more basal node. This fact also seems to me to offer difficulties to the hypotheses of dominance by means of inhibiting or by means of nutritive substances. On the other hand, it is apparently an expression of the physiological gradient, the primarily quantitative gradation in physiological condition along the plant axis, which, as I believe, is the basis of physiological correlation in the plant. In other words, the relation of dominance and subordination is also an expression of the physiological gradient, and the movements of substances in the plant are not the primary factors in physiological correlation, but rather the consequences of the differences which constitute the physiological axial gradient.

WATER DEFICIT AND THE ACTION OF VITAMINES, AMINO-COMPOUNDS, AND SALTS ON HYDRATION[1]

D. T. MacDougal

(Received for publication January 17, 1921)

It is well known to any one who has seriously examined cells in a living condition that protoplasm is a viscous substance which at different times or in different parts of the same protoplast may vary in consistency from a liquid to that of a firm jelly, a behavior characteristic of an emulsoid colloid. This term in the present instance is applied to substances which in a condition of hydration exist in two distinct phases in the mass; in one, a few or many molecules of the solid substance are combined or held together by adsorption with a relatively small number of water molecules to form a denser phase, which in the more liquid condition of the mass floats in or is surrounded by a more fluid phase in which the solid particles sustain a much smaller proportion to the water.

If we begin to form our picture of the colloid in this condition, which would be that of melted agar or gelatine, we shall be ready to follow its transformation to that of a jelly by visualizing an action by which the surface tension of the aggregates of molecules is increased by lowered temperatures or other causes, the aggregates coming together to form meshworks or honeycombs, running through or partially enclosing the material of the more liquid phase. The possibilities implied in the reversal of these phases, important as they may be, may be disregarded during the present discussion.

Living matter is anything but such a simple substance. The results of all of our examinations by physical and chemical methods are to the effect that the living matter of plants includes the following groups of substances which may assume the colloidal conditions described above: First, the nitrogenous substances, which include not only albumin and all its derivatives, but also synthetic compounds. Next in importance, and constituting perhaps the greater part of the mass, are the pentosans or mucilaginous sugars which may be formed in carbohydrate metabolism in any part of the mass when hexoses are converted into pentoses and these are condensed into the pentosans by dehydration. The presence of such fatty acids as stearic, palmitic, and oleic, and the readiness with which they form soaps by combination with potassium, sodium, and magnesium, make it also certain that such substances are an invariable component of the biocolloids.

---

[1] Invitation paper read before the Physiological Section of the Botanical Society of America, in the symposium on biophysics, at Chicago, December 28, 1920.

In addition, a certain amount of lipins may be present, but as their possible hydration action in the colloidal mass must be very slight, they may be left for consideration in a discussion of other phases of protoplasmic action.

When the general properties of these main components of living matter are reviewed, it is seen that the albuminous or proteinaceous substances are amphoteric, behaving as either acids or bases according to the hydrogen-ion concentration of the solution, that they generally unite with the highest proportion of water when in an acidified condition, and that their hydration is also facilitated in lesser degree by the action of bases and their salts. The pentosans or mucilages are weak acids and undergo the highest degree of hydration in neutral or slightly alkaline solutions, in extremely dilute solutions of the common salts of potassium, magnesium, calcium, and sodium, or in the presence of certain amino-compounds described in previous papers. That the nitrates, chlorides, and sulphates of these metals may also increase the hydration capacity of the pentosans will be demonstrated in the present paper.

The soaps are characterized by their capacity for forming films, and their high hydration capacity is altered to such degree by variations in the hydrogen-ion concentration of the solutions as to make them "sensitizers" as to acidity in any colloidal mass into which they may enter. It is to be noted that the range of acidity or alkalinity of interest to the physiologist in the present connection is that which lies between the measurements expressed by the symbols $PH = 3$ and $PH = 11$, and that of the salts as chiefly between 0.001 and 0.0001 M.

Taking into consideration the complex conditions suggested above, it is reasonable to infer, since we have as yet devised no means of direct observation, that, as the more complex proteins and the pentosans do not dissolve or diffuse in each other, they form separate aggregates, and that the sponge or meshwork of the solidified biocolloid or protoplasm must therefore consist of an interwoven meshwork of the two. This inference may not be carried safely beyond a certain point, however, nor to imply that no nitrogenous substances of the cell may engage, or be adsorbed by, the pentosans, since the results of the action of histidine, glycocoll, alanine, asparagine, and phenylalanine upon agar go far to suggest the possibility of such unions. The soaps which may be present in the cell colloids are not known to form combinations with the carbohydrates, and, although some uncertainty exists as to what disturbances may be caused by their contacts with the proteins, yet it may be assumed for the present that they form films enclosing the more solid phase of the double meshwork, being thus an interphase in the colloidal machine, highly sensitive to the action of the hydrogen-ion and adding greatly to the complexity of the possible action of the mass as a whole in response to various solutions and environmental conditions.

In this my results are in accord with those of Dr. Clowes, who ascribes variations in permeability to effects of electrolytes and metabolic products on interfacial soap films.[2]

Referring to the picture of the colloidal mechanism described above, it is obvious that, while the proteins or albumins and the pentosans must form separate strands or plates, the smaller aggregates in the more liquid portion or phase mingle with each other, and it might also be suggested that various unions might take place between molecular groups of pentosans with amino-compounds or salts, and that actual salts might be formed by the albumins when the colloid is immersed in hydroxides or salts such as those of potassium, sodium, or magnesium. Here then we have a crude statement of a theory of the colloidal condition of protoplasm upon which extensive experimentation as to the action of bases, salts of common metals, acids, amino-compounds, and vitamines has been carried on with the acquisition of results of positive value. These reactions are our real aim, and after we have crossed our bridge of hypothesis to the solid ground of facts, the fate of the bridge which may have served us well is of minor importance.

The arrangement of living matter inferred has much to support it. It is one in which the separate components of the colloidal machine each present a separate and individualized capacity for hydration changes under any set of conditions or at any given temperature for example, and when immersed in water would move with differentiated speed to saturation or satisfaction.

If the water in which the colloid is immersed holds substances in solution the ions of which may form combinations with the main components mentioned above, their capacity for holding water in combination may be altered, and combinations or splittings in the metabolism of substances in protoplasmic colloids may also exert such action. Thus, a dissociation resulting in the freeing of hydrogen ions tends to increase the hydration capacity of the protein strands or aggregates of the mass might lessen that of the pentosans and soaps. Slightly acid amino-compounds as glycocoll would increase the hydration of the pentosans while exerting practically no effect on the albumin or albumin derivatives. It is evident without further elaboration that in the albumin-pentosan-soap machine we have a mechanism capable of an almost endlessly diversified action in swelling and growth.

With so much prelude we may now advantageously turn to a consideration of recently acquired results obtained by testing the action of salts, balanced solutions, amino-compounds, and vitamines on such colloidal mechanisms and on biocolloids and cell masses, living and dead.

In an earlier paper[3] I had advanced the idea that the common metals which enter into nutritive solutions, as potassium, magnesium, sodium, and calcium, might find their chief importance in restricting, limiting, or defining hydration. Such an action is exerted by these bases in the form of hydroxides when tested at 0.01 N. MacDougal and Spoehr[4] found later that the hydroxides of the strong metallic bases limit the hydration of agar according to their position in the electromotive series, the least swelling taking place under the action of the strongest base at concentrations of 0.01 N, with the apparent exception of rubidium. Beginning with the strongest, the series runs $K > (Rb) > Na > Li$. The various effects of barium, calcium, and strontium are not so clearly determined, and the quantitative relations of these metals are not known definitely. Hydration values of agar at 0.01 N were $Sr(OH)_2 = 815$, $Ca(OH)_2 = 860$, $Ba(OH)_2 = 900$.

---

[2] Clowes, G. H. A. On the action exerted by antagonistic electrolytes on the electrical resistance and permeability of emulsoid membranes. Proc. Soc. Exper. Biol. and Med. **15**: 108. 1918.

[3] MacDougal, D. T. Growth in organisms. Science, **49**: 599–605. 1919. (See p. 11 of reprint).

These concentrations are far beyond the actual range of conditions in the cell, however, and when reduced concentrations were used it was seen that hydration of agar in calcium hydroxide exceeds that in water at 0.0001 N of the hydroxide, and this effect is also produced at 0.00001 N. Increase of hydration beyond that of water by dilute solutions of hydroxides of calcium, potassium, rubidium, potassium, sodium, and lithium, and excess values for aniline and ammonium hydroxides were obtained.

It was also seen that the strongest of the bases, potassium, in the form of a hydroxide would increase the swelling of agar-albumin mixtures to a point beyond that taking place in water alone.

The next logical step was to test the effects of salts of the common metals on swelling of the biocolloidal components. Here again the interesting fact was found that as chlorides, sodium and potassium at 0.001 M caused greater hydration of agar than water, the swelling being greater in the potassium. At 0.0001 M, sodium, potassium, magnesium, and calcium chlorides caused greater swelling than in water, the maximum swelling being in sodium, the next in potassium, and the least in calcium.

When chlorides of sodium and potassium were tested in series as shown in tables 1 and 2, it was found that pentosan-albumin mixtures showed increased hydration in the potassium chloride only as indicated in the tables.

TABLE 1. *Hydration of mixtures of agar 3 parts, gelatine 2 parts at 14° C. Plates 0.18 mm. in thickness; swelling of sections given in thickness and volume*

	0.01 M		0.001 M		0.0001 M.	
	Th.	Vol.	Th.	Vol.	Th.	Vol.
HCL	550	600	930	1,025	1,430	1,575
KCl	1,900	2,015	2,270	2,530	2,440	2,640
CaCl	920	960	1,220	1,345	2,030	2,268
Water					2,200	2,330

---

[4] MacDougal, D. T., and Spoehr, H. A. The components and colloidal behavior of plant protoplasm. Proc. Amer. Phil. Soc. **59**: 154. 1920.

TABLE 2. *Hydration of mixtures of gelatine 3 parts, agar 2 parts at 14° C. Plates 0.18 to 0.19 mm. in thickness; swellings given in thickness and volume*

	0.01 M		0.001 M		0.0001 M	
	Th.	Vol.	Th.	Vol.	Th.	Vol.
HCl	1,200	1,320	650	690	860	920
KCl	800	880	900	1,010	1,620	1,850
$CaCl_2$	710	740	870	940	1,300	1,430
Water					1,275	1,420

The agar-gelatine mixtures are seen to have a higher capacity than the gelatine-agar, and also to react by high hydrations in greater concentration of potassium.

Up to this point colloids including only two of the supposed main elements in protoplasm have been used. An agar-gelatine mixture was now made to which was added a thousandth part of a soap which is probably nearly all sodium stearate. The results of the hydration swellings are given in table 3.

TABLE 3. *Hydration of plates of agar 3, gelatine 2, Ivory soap 0.005 g. at 15° C. Swellings given in thickness and volume*

	0.01 M		0.0002 M		0.0001 M	
	Th.	Vol.	Th.	Vol.	Th.	Vol.
NaCl	890	1,020			2,330	2,600
Balanced solution (Na 50 : Ca 1)	810	842			2,460	2,660
$CaCl_2$	850	920	1,200	1,374	2,600	2,940
KCl	970	1,050			2,750	2,050
HCl	1,280	1,460	1,130	1,270	1,200	1,250
Water					1,500	1,700

This biocolloid, representing more nearly the colloidal constitution of living matter, was seen to have higher hydration capacity in all salt solutions, and to have such capacity lessened in even the very dilute acid. A similar preparation in which the soap was pure potassium oleate gave results less marked as to the action of the salts, but the increase in the balanced solution was proportionately much greater, and the retarding effect in acids much greater. Ample justification exists, therefore, for a correction of the earlier statement as to the effect of salts of the common metals on biocolloids which have been found to offer many profitable analogies to living material.

The correction implies that we may confidently look to these salts as accelerators of hydration and growth, or as increasing the water deficit of living matter.

Lastly we come to the amino-acids and to water-soluble vitamine. I have previously pointed out in many papers that the commoner amino-acids, glycocoll, alanine, phenylalanine, asparagine, and histidine, which have been proved to promote growth, also accelerate hydration in biocolloids.

As an additional step in this work, the effect of the water-soluble B yeast vitamine on biocolloids and living and dead cell-masses was measured. Solutions of this material at one tenth percent, having an acidity of PH = 5.25 as determined by the colorimeter method, were used, and measurements were taken by the auxograph. Taking the swelling in water as 100, the values obtained in the vitamine were as below:

Potato tubers, young, sections	75
Potato tubers, large, sections	230
Squash fruits, young, sections of pulp	110
Squash fruits, mature, sections of pulp	115
Orange seedlings, root tips, living	150
Orange seedlings, root tips, dried	120
Corn root tips, small, living	88
Corn root tips, large, living	78
Corn root tips, large, dried	180
Strawberry root tips, living	133
Sunflower stems, sections of pith, mature, living	150
Opuntia, sections of young joints	94
Opuntia, sections of mature joints	170
Opuntia, dried slices	90
Agar	140
Agar and soap	132
Agar and potassium oleate	80
Agar and phenylalanine	95
Agar 3, gelatine 2, and "salts"	130
Agar 3, gelatine 2, soap	92
Agar 3, gelatine 2	136
Agar 3, gelatine 3	135
Agar 2, gelatine 3	143
Agar 2, gelatine 3, and "salts"	130
Gelatine	163
Gelatine and soap	92

The vitamine is seen to increase the hydration swelling = water capacity or water deficit of agar, agar-soap, agar-gelatine, agar-gelatine-salts, and gelatine, but to lessen hydration in some biocolloids containing soaps which would be sensitive to the free hydrogen ions of the vitamine solution. Parallel action in living and dead cells is implied, although it is to be noted that such cells may already include a certain amount of their characteristic vitamines and that the added vitamine could exert no additional effect except that of reducing hydration capacity.[5]

SUMMARY

1. The theory as to the constitution of living matter by which plant protoplasm is taken to be a pentosan-albumin-soap colloidal mixture is

---

[5] MacDougal, D. T. The effects of yeast vitamine water-soluble B on plant cell-masses and on biocolloids. Proc. Soc. Exper. Biol. and Med. 18 : 85. 1920.

briefly restated. Its adequacy at the present stage of experimentation is confirmed.

2. The metals which form the bases of nutrient salts, as hydroxides and as chlorides and nitrates, are found to increase the hydration capacity, or the water deficit of the principal components of biocolloids, and of biocolloids of certain composition.

3. Biocolloids containing soaps show a high degree of sensitiveness to hydrogen ions, or acidity. Such biocolloids show marked action in balanced solutions of sodium and calcium as shown by data too detailed to be given in this paper.

4. Yeast vitamine water-soluble B, in a solution slightly acid, increases the swelling, hydration, or water deficit in some living and dead plant cell masses, and lessens it in others. Similar diverse action on biocolloids was found.

5. All of the substances tested which are known to facilitate growth in plants are found to increase hydration capacity or water deficit in some of the test objects. The list includes chlorides and nitrates of sodium, potassium, magnesium, and calcium in various concentrations between 0.001 N and 0.0001 N, glycocoll, alanine, phenylalanine, histidine, and water-soluble B yeast vitamine. Hydroxides of sodium, potassium, lithium, rubidium, calcium, ammonium, and aniline also increase hydration values in some of the components of living matter.

THE EUSPORANGIATE FERNS AND THE STELAR THEORY

D. H. Campbell

(Received for publication January 17, 1921)

Some thirty years ago, as the result of an extensive series of investigations, Van Tieghem, the distinguished French botanist, brought forward his stelar theory which offered an interpretation of the nature of the tissue systems of the higher plants quite different from that which had been held by Sachs, de Bary, and other earlier investigators.

Van Tieghem's views, with some more or less important modifications, have been pretty generally accepted by the morphologists of the past two decades, and have been assumed to apply to all the vascular plants.

Among the forms which have been the subjects of frequent and detailed study in regard to the nature of their stelar structures are various ferns. These studies have been directed largely toward the elucidation of the evolution of the stelar structures of the Filicineae and have included a study of many fossil ferns as well as the existing types. These investigations have thrown much light upon the relationships existing between the many Palaeozoic fern-like plants and their living relatives.

An extensive literature on the subject has grown up in the past twenty years, especially in England, and with it a somewhat elaborate terminology based upon the assumption that the fibro-vascular skeleton of the fern stem is a strictly cauline "stele" with which the corresponding foliar bundles are simply connected by the so-called "leaf-traces."

The general acceptance of Van Tieghem's stelar hypothesis and its modification by Strasburger and other investigators, especially in England, are sufficiently familiar to students of plant anatomy. Van Tieghem concerned himself chiefly with the Spermatophytes, and his interpretation of the stelar structures of the ferns has been a good deal modified by the English investigators and by Jeffrey in this country. The latter[1] has summarized these conclusions, and this has also been done at length by Bower[2] and Schoute.[3]

Some of the most recent contributions to the subject[4] apparently accept these views as applying universally to the ferns, and quite ignore the evidence brought forward by the writer nearly ten years ago,[5] and amply verified by the more recent work of West,[6] that the stelar theory, as usually understood, cannot be reconciled with the facts as revealed by a study of the Eusporangiatae. The writer has therefore thought it worth while to summarize this evidence, and also to add further facts derived from a recent study of Botrychium.

---

[1] Jeffrey, E. C. The structure and development of the stem in Pteridophyta and Gymnosperms. Philos. Trans. Roy. Soc. B, **195**: 119–146. 1902.

[2] Bower, F. O. The origin of a land flora. London, 1908.

[3] Schoute, J. C. Die Stelär-Theorie. Jena, 1903.

[4] *E.g.*, Thompson, J. M. New stelar facts, and their bearing on stelar theories for the ferns. Trans. Roy. Soc. Edinburgh **52**: part 14, no. 28. 1920.

[5] The Eusporangiatae. Carnegie Inst. Washington Pub. 140. 1911.

From an extensive series of investigations on nearly all the genera of eusporangiate ferns, the writer was forced to the conclusion that a cauline stele is either completely wanting in these ferns, or that, where cauline stelar tissues are present, they constitute an insignificant part of the fibro-vascular skeleton. West's studies on the Marattiaceae confirm these conclusions, which, however, as already indicated, seem to have been quite overlooked by some of the recent investigators.

According to Van Tieghem's view, most of the ferns are "polystelic," the individual strands of the net-like woody cylinder of the stem being considered to be of independent origin, the reticulate structure resulting from the coalescence of these independent "steles." Most of the later students of the ferns consider the "dictyostele," or reticulate woody cylinder, to be a single structure, *i.e.*, the stem is regarded as "monostelic," the openings being designated "leaf-gaps" where the leaf-traces join the cylindrical cauline stele. In nearly all the recent studies on the stelar structures of the ferns, the strictly cauline nature of the axial fibro-vascular tissues is apparently taken for granted.

Brebner[7] first pointed out that in the very young sporophyte of *Danaea simplicifolia* the primary fibro-vascular bundle is common to the cotyledon and root, and that for a considerable time there is no evidence of any cauline stele. Little attention has been paid to these facts by most recent students of the ferns, but in a recent paper by West[8] the accuracy of Brebner's conclusions has been fully recognized.

The writer's attention was first called to the real state of affairs in the Eusporangiatae as the result of a study of the embryology of *Ophioglossum Moluccanum.*[9]

Many years ago, Mettenius[10] described the young sporophyte of *Ophioglossum pedunculosum* as consisting at first simply of a leaf and root, the definitive sporophyte arising secondarily as a bud upon the primary root. Very little attention was given by later students of the Ophioglossaceae to this really remarkable discovery, and it has been either forgotten or ignored.

The writer collected in Java a considerable number of young sporophytes of *O. Moluccanum* Schlecht, which is possibly identical with *O. pedunculosum* Desv. Specimens were secured in Ceylon of *O. reticulatum* L. or some closely related species. These agreed closely with the species described by Mettenius, and indicated that in these tropical species of Euophioglossum, the young sporophyte is absolutely destitute of any cauline tissues, being composed at first of a simple primary leaf, or cotyledon, which merges insensibly into the root (fig. 1). A single central fibro-vascular bundle or "stele" extends without interruption from the petiole into the root, and its structure is essentially the same throughout, *viz.*, "collateral" in the petiole, "monarch" in the root.

---

[6] West, C. A contribution to the study of the Marattiaceae. Annals of Bot. **31**: 361–414. 1917.

[7] Brebner, G. On the prothallus and embryo of *Danaea simplicifolia,* Rudge. Annals of Bot. **10**: 109–122. 1896. On the anatomy of Danaea and other Marattiaceae. Annals of Bot. **16**: 517–552. 1902.

[8] *Loc. cit.*

[9] Studies on the Ophioglossaceae. Ann. Jard. Bot. Buitenzorg II, **6**: 138–194. 1907.

[10] Mettenius, G. Filices Horti Botanici Lipsiensis. Leipzig, 1856.

Mettenius does not describe in detail the origin of the different organs of the embryo sporophyte. In *O. Moluccanum* the writer found that the sporophyte at a very early stage consists of but two portions, a large basal foot and an apical conical portion developing subsequently into the cotyledon. At this stage, the embryo is strongly suggestive of that of Anthoceros. The growing point of the primary root arises endogenously, being formed near the center of the embryo where the base of the young cotyledon

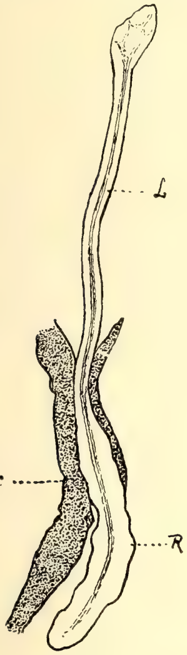


FIG. 1.

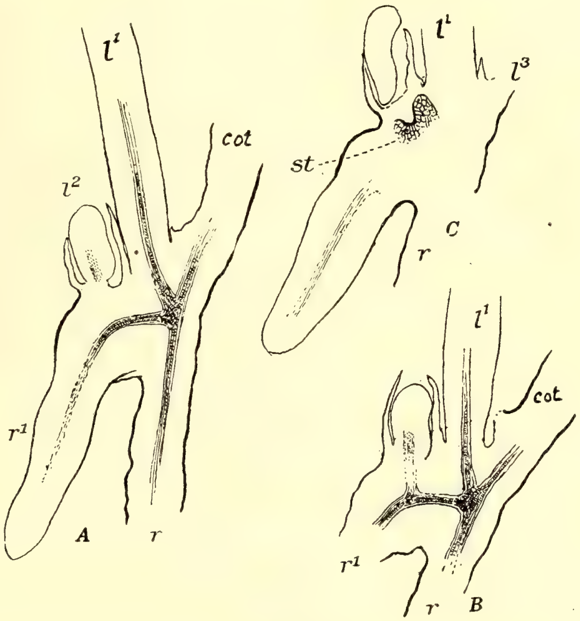


FIG. 2.

FIG. 1. Median longitudinal section of a young sporophyte of *Ophioglossum Moluccanum*, showing the primary stele traversing the cotyledon, *L*, and the root, *R*. *Pr*, the gametophyte.

FIG. 2. Three longitudinal sections of the bud developed upon the primary root, *r*, of *Ophioglosum Moluccanum*. *cot*, cotyledon; l^1, l^2, the first two leaves of the bud; r^1, the first root of the bud; *st*, the stem apex.

joins the foot. As the root grows, it pushes downward through the foot, which is practically eliminated and is no longer recognizable. The young sporophyte is thus bipolar in structure, and the stele, as we have seen, is continuous through the cotyledon and root.

It is not until the cotyledon and primary root are fully developed that the bud which is to develop into the definitive sporophyte first becomes evident. This begins as a group of meristematic cells close to the stele of the root—exactly in the way a secondary root arises. The first two leaves of this bud are formed quite independently of the apical meristem of the young bud. The stele of the first leaf of the bud joins directly with the stele of the primary root, while that of the second leaf is joined to the base of the stele of the first root developed from the bud (fig. 2).

It is thus clear that the fibro-vascular system in the sporophyte of *O. Moluccanum* begins as a single continuous strand extending from the petiole of the cotyledon into the primary root and practically of the same structure throughout, the xylem and phloem of the "collateral" foliar portion being continuous with the corresponding tissues of the "monarch" root portion. This primary "stele" is not a "protostele," *i.e.*, it is not "concentric" in structure, and, moreover, it is not a cauline structure.

A study of the older sporophyte shows that much the same condition prevails as in the earlier stages. The leaf-traces unite with root bundles and with the older leaf-traces, and there is thus built up the open "dictyostele" found in the adult rhizome.[11] There is no indication of the development of any stelar tissues except those belonging either to the leaves or to the roots.

Botrychium

In *Botrychium Virginianum* the structure of the axial stele is quite different from that in Ophioglossum. At an early stage,[12] the axis of the young sporophyte shows an almost unbroken cylindrical stele enclosing a central pith instead of the large-meshed "dictyostele" of Ophioglossum. In the very young sporophyte a single strand of procambium extends through the axis of the cotyledon into the primary root; but as these organs usually make a marked angle with each other, the primary vascular strand is strongly bent instead of being straight, as it is in *Ophioglossum Moluccanum.* Very soon the second leaf is developed, and a similar vascular strand is formed in it, which unites with the primary vascular bundle near the point of junction between the petiole of the cotyledon and the base of the primary root (fig. 3), and the fusion of the three bundles appears as a closed ring in cross section.

The traces from the later leaves behave in much the same way, and the massive "siphonostele" found in the older stem is thus built up.

---

[11] For details see "The Eusporangiatae," pp. 89–93.

[12] Jeffrey, E. C. The gametophyte of *Botrychium Virginianum.* Trans. Canad. Inst. 5: 1–32, 1898.

As in Ophioglossum, the bundles are collateral, and, as is well known, there is developed a cambium between xylem and phloem, and also medullary rays, so that the structure of the woody cylinder in the older sporophyte is extraordinarily like that in the Gymnosperms and in many Dictoyledons.

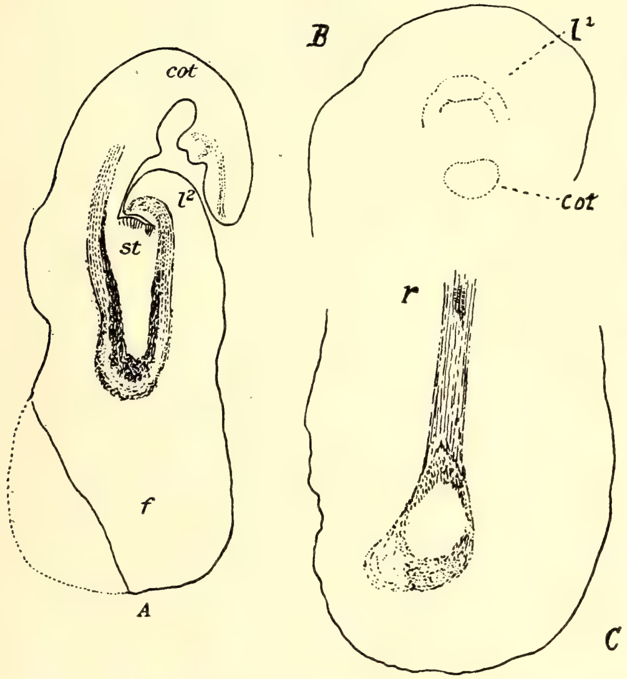


Fig. 3. *A*. Median longitudinal section of a young sporophyte of *Botrychium Virginianum*, cut at right angles to the primary root. The two leaf-traces unite at the junction of the root. *B*. Transverse section of a similar sporophyte showing the two leaf-traces. *C*. Another section of the same sporophyte showing the junction of the two leaf-traces with the stele of the root.

As in Ophioglossum, there is also in Botrychium no evidence of any procambial tissue in the axis above the youngest leaf-trace, *i.e.*, the stelar tissues as in Ophioglossum are composed entirely of the coalescent leaf-traces.

A still closer resemblance to Ophioglossum is shown by the young sporophyte of *B. obliquum* Mühl., which the writer has recently examined. In this species the cotyledon and primary root, instead of forming an angle with each other, as in *B. Virginianum*, have a common axis, and the orientation of these organs is like that in *Ophioglossum Moluccanum*. The writer is indebted to Dr. H. L. Lyon of Honolulu for the material upon which his investigations were made, and for the accompanying photograph (fig. 4).

Dr. Lyon[13] first directed attention to the peculiarities of this species,

[13] Lyon, H. L. A new genus of Ophioglossaceae. Bot. Gaz. **40**: 455–458. 1905.

and through his courtesy the writer has been able to examine a large number of preparations made by Dr. Lyon, as well as to make a series of slides from gametophytes and sporophytes furnished by him.

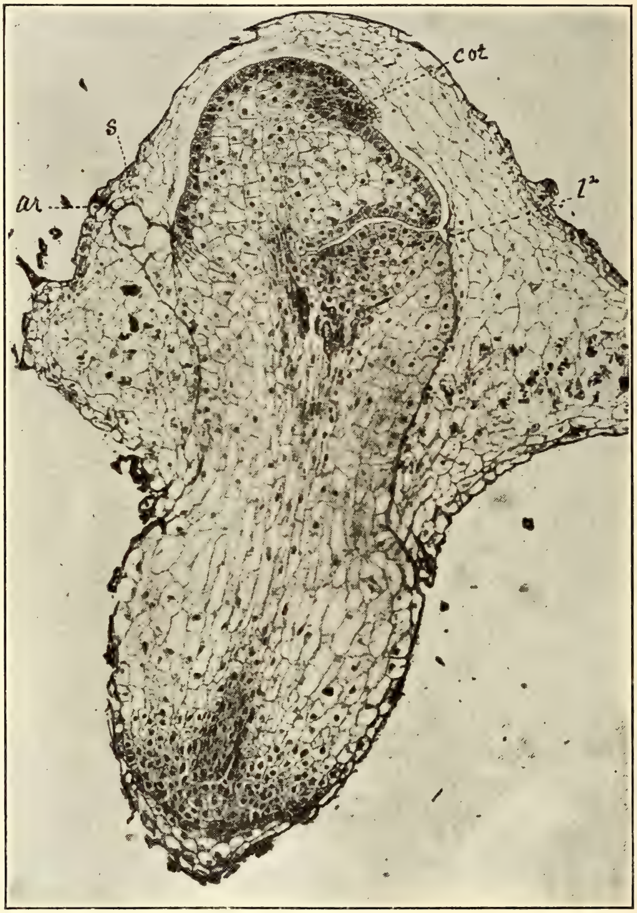


FIG. 4. Median longitudinal section of a young sporophyte of *Botrychium obliquum*, showing the continuity of the steles of the cotyledon and root, and the junction of the two leaf-traces at the base of the root. Photograph by Dr. H. L. Lyon.

In *B. obliquum* (fig. 4), there is a conspicuous suspensor, comparable to that in Danaea[14] and Macroglossum.[15] Moreover, the young sporophyte is bipolar in structure, and the relation of cotyledon and root is essentially the same as in *Ophioglossum Moluccanum*. As in the latter, the primary root of *Botrychium obliquum* is endogenous in origin, instead of being superficial as it is in *B. Virginianum*. It grows downward through the foot, exactly as it does in *Ophioglossum Moluccanum* and the Marattiales, and the foot thus is practically obliterated, instead of forming a large part of the embryo as it does in *B. Virginianum.* In the latter, as we have seen, the primary root and the cotyledon make a sharp angle with each other, while in *B. obliquum* their axes are in a straight line, and the common vascular bundle, or stele, closely resembles that of *Ophioglossum Moluccanum.* The stele of the primary root, however, is diarch as it is in *B. Virginianum* and in most of the Marattiales.

---

[14] Campbell. The Eusporangiatae.

[15] Campbell, D. H. The structure and affinities of *Macroglossum Alidae*, Copeland. Annals of Bot. **28**: 651–669. 1914.

The development of the cylindrical stele in the axis of the young sporophyte is essentially the same as in *B. Virginianum, i.e.*, it is formed by the union of the broad leaf traces of the early leaves.

The writer was not able to obtain the youngest stages of the sporophyte in Helminthostachys, but from a study of somewhat older specimens the conclusion was reached that the stele is formed in much the same way as in Botrychium.[16]

The Marattiales

Brebner[17] first called attention to the absence of a cauline stele in the young sporophyte of *Danaea simplicifolia*, although the writer[18] had shown in an earlier study on *Marattia* that there was at first a continuous procambium strand traversing the young cotyledon and primary root. Farmer's figures of the young sporophyte of Angiopteris[19] show the same condition. The most recent contribution to the subject is a paper by West[20] who finds that there is no cauline stele in the young sporophyte of the Marattiaceae.

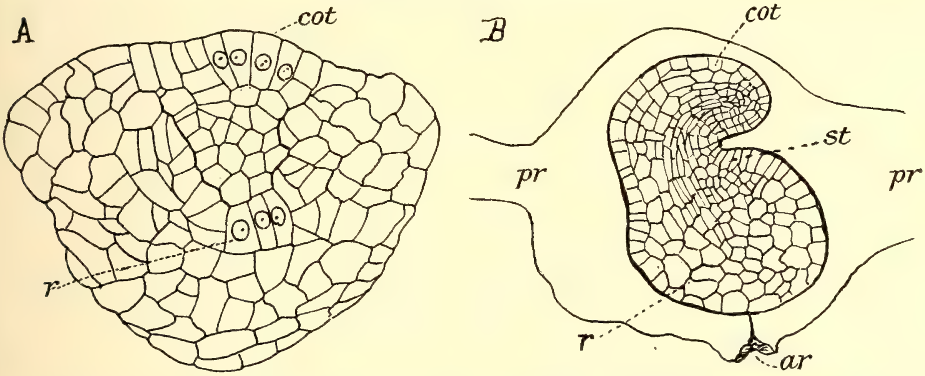


Fig. 5. *A*. Vertical section of an embryo of *Danaea elliptica*, passing through the cotyledon, and showing the endogenous origin of the root, *r*. *B*. A nearly median section of a very young sporophyte of *Marattia Douglasii*, showing the young stele extending from the cotyledon into the root. The root apex does not show in this section.

---

[16] Campbell. The Eusporangiatae.

[17] Brebner, *loc. cit.*

[18] Campbell, D. H. Observations on the development of *Marattia Douglasii*, Baker. Annals of Bot. **8**: 1–20. 1894.

[19] Farmer, J. B. The embryogeny of *Angiopteris evecta*, Hoffm. Annals of Bot. **6**: 265–270. 1892.

[20] West, *loc. cit.*

The writer[21] examined with great care the development of the fibrovascular system in the young sporophytes of *Danaea Jamaicensis* Underw. and *D. elliptica* Smith, and also the younger stages of the sporophytes of species of Angiopteris, Kaulfussia, and Marattia. In all of these (fig. 5, *A*) it was found that the primary root is deep-seated, growing through the foot precisely as it does in *Ophioglossum Moluccanum* and *Botrychium obliquum*, and there is a single primary stele traversing the axis of the cotyledon and root (fig. 5, *B*). The young plant is thus bipolar, so far as the cotyledon and root are concerned, and the insignificant stem apex appears as a small lateral appendage near the junction of the two primary organs. No procambium can be made out in the very small mass of tissue which can be assigned to the stem.

This is particularly well shown in Danaea (fig. 6), where the stem apex is seen in the angle formed by the junction of the bundle from the second leaf with the primary bundle, but no sign of procambial tissue can be seen in the region above this junction.

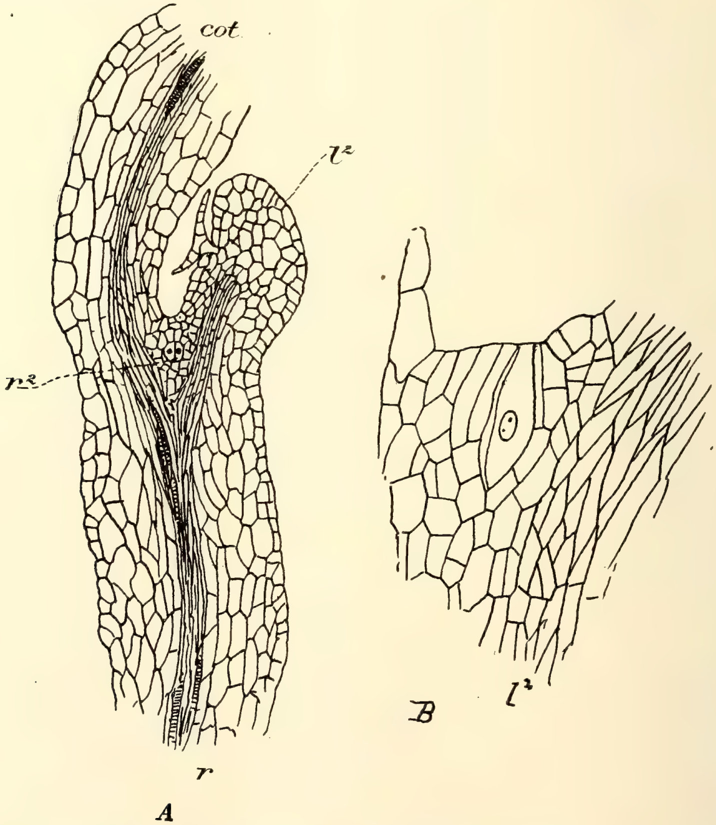


FIG. 6. *A*. Longitudinal section of young sporophyte of *Danaea elliptica*, showing the junction of the two leaf-traces. *B*. The stem apex of the same sporophyte with the trace of the second leaf, l^2, passing to one side of it.

[21] Campbell. The Eusporangiatae.

The vascular strands of the first two leaves are collateral in structure, and after their fusion near the junction of the cotyledon and primary root, the xylems of the two bundles are easily recognizable and are continuous with the two xylems of the diarch root (fig. 7).

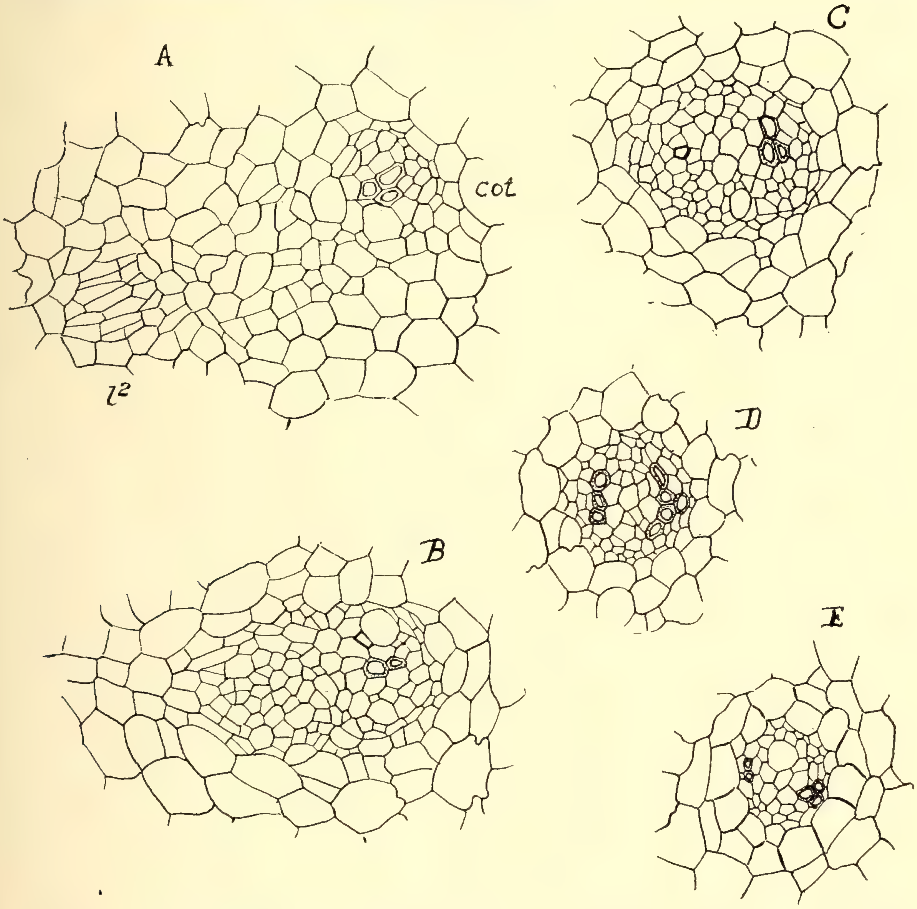


FIG. 7. Five cross sections of a series from a young sporophyte of *Danaea Jamaicensis*. *A* shows the two primary leaf-traces; *B* and *C*, the fusion of these to form the solid stele of the young plant; *D*, the transition region of the sporophyte; *E*, the stele of the root.

It is clear that the central region of the young sporophyte is not strictly a cauline structure, since the cotyledon and primary root are in no sense appendages of the stem, which at this stage consists only of the very small area in the immediate vicinity of the apical meristem. Moreover, the foot contributes a considerable amount of the outer tissue in the central region of the young sporophyte.

As the new leaves are formed, close to the stem apex, their steles unite with those of the older ones, and there is thus built up a "dictyostele," much like that in Ophioglossum. After about seven leaves have been formed, this structure is complicated by the development of vascular strands inside the dictyostele, and these "commissural" strands can be traced up to the apical meristem of the stem, and are therefore true cauline strucures. As the sporophyte increases in size, the number of leaf-traces increases and further commissural strands are also formed, but the greater part of the elaborate skeleton of the adult sporophyte is undoubtedly of foliar origin, only the relatively unimportant commissural strands being cauline.

The history of the development of the fibro-vascular skeleton of the Eusporangiatae leads inevitably to the conclusion that in the Ophioglossales the whole stelar system is derived from the leaves and roots, and this is true to a great extent for the Marattiales, although in the latter the commissural strands are really cauline in origin.

It may be said also that the cortical tissue of the caudex is to a considerable extent of foliar origin, being made up of the coalescent leaf bases. This is, to some extent, a confirmation of Delpino's theory that the leaves, instead of being appendages of the stem, are the primary organs, and that the so-called stem is formed by the coalescence of leaf bases.[22]

It is also clear that the medullary tissue is in no case of stelar origin, but is always a portion of the ground tissue (to use the older term) which is more or less completely enclosed by the coalescent foliar steles.

The great preponderance of the foliar structures over the stem in most Filicineae has not received the attention that might be expected, in the many discussions on the nature of the stelar tissues that have appeared. Few of the higher plants show this to the same extent, and it may be questioned whether any Angiosperm can show leaves equal in complexity to such ferns as Angiopteris and some of the tree ferns. It is true that the leaves of some palms are bulkier, but structurally they are decidedly simpler. So far as mere length is concerned, probably some species of Gleichenia and Lygodium surpass even the longest palm leaf. Hooker[23] states that *Lygodium articulatum* A. Rich has stipes 50 to 100 feet in length arising from a slender prostrate rhizome.

The very young sporophyte of Ophioglossum, which the writer believes to be the most primitive of existing ferns, has no stem at all, but consists simply of a single leaf and root, the stem arising secondarily as an adventitious bud. This is entirely in harmony with the theory of the derivation of the Filicineae from Anthoceros-like ancestors; and the predominance of the leaf, shown in the young sporophyte, is maintained throughout the whole history of the Filicineae.

The assumption, therefore, that the stem is the predominant or primary organ of the sporophyte, and that the leaves are mere appendages of this, is hardly borne out by a study of the ontogeny, at least of the Eusporangiatae; and this probably will be shown also to be the case in many, at least, of the Leptosporangiatae.

---

[22] See Schoute, *loc. cit.*, p. 97.

[23] Hooker, J. D. Handbook of the New Zealand flora, p. 385. London, 1867.

When one compares the slender rhizome of such ferns as Lygodium, Gleichenia, and many Hymenophyllaceae with the large and highly developed leaves, it may well be questioned whether the leaves should be regarded as mere appendages of the relatively insignificant axis. This is particularly the case with such forms as Gleichenia and Lygodium whose leaves show almost unlimited power of continuous growth in length.

It is very important that further studies upon the origin of the stelar tissues of the Leptosporangiatae should be undertaken. Most of the studies already made upon the ontogeny of these ferns have not dealt with the earliest stages of the stelar tissues, but have started with the fully developed stele of the young sporophyte, assuming that this axial stele is really of cauline origin and not a composite structure derived from a fusion of leaf-traces. In order to solve this question it is necessary to examine series of sections, both transverse and longitudinal, including the growing point of the stem and the adjacent regions. In longitudinal sections alone there is danger of misinterpretation, as the traces of the youngest leaves may be easily mistaken for a true cauline stele; but if corresponding transverse sections are examined, it is then possible to determine whether or not there is a stele of strictly cauline origin.

It will not be surprising if such a test applied to the Gleicheniaceae and Hymenophyllaceae will show that the solid or tubular axial steles are in reality composed of coalescent leaf-traces as they are in Botrychium and in the young sporophytes of some of the Marattiales.

It is very desirable that the many careful studies of the stelar tissues of the ferns be reviewed to determine whether the usual interpretations of the relation of the tissues of the leaves and axis are tenable. A satisfactory solution of the problem necessitates an examination of the origin of the tissues in the young sporophyte as soon as it emerges from the gametophyte, and a further study of the building up of the stelar structures as new leaves are developed, tracing the origin of the individual vascular strands to their beginning. Reconstructions from series of cross sections of the completed bundles of older stages will not suffice.

Conclusion

The presence of a single cauline stele—"protostele," "siphonostele," "dictyostele"—is not borne out by the history of the stelar tissues in the Ophioglossales and Marattiales. In all of these the stelar system begins as a single strand common to the first leaf and root; the stem apex arises adventitiously in *Ophioglossum Moluccanum* and *O. pendulum*, and is very insignificant in Botrychium and the Marattiales. No procambium is developed in the stem region in the young sporophyte.

In the Ophioglossales, the stelar structures of the axis are built up exclusively of leaf-traces to which the bundles of the roots are joined. This condition obtains also for the earlier stages of the Marattiales, but is complicated later by the formation of "commissural" strands, which are of cauline origin.

The "dictyostele" of Ophioglossum and most Marattiales is in no sense a monostele. The "foliar gaps" are not breaks in a single tubular stele, but are merely spaces between the coalescent leaf-traces, and the pith is part of the ground tissue included within the cylindrical network formed by the united bundles derived from the leaves.

In short, the condition found in the axis of the eusporangiate ferns is more in accord with the older theory of "common" bundles traversing a ground tissue, and united to form the woody cylinder of the axis, than with the assumption of a true cauline stele.

The condition existing in the eusporangiate ferns by no means implies that the stelar hypothesis must be completely discarded. There seems to be no question of its application to the Lycopods, Conifers, and many Angiosperms; but in all of these, the relative importance of stem and leaf is very different from the condition in the ferns; and it will not be surprising if, when the different types of the Leptosporangiatae are subjected to a thorough examination of the origin of the stelar tissues in the young sporophyte, it will be found that in these, as well as in the Eusporangiatae, the axial stelar tissues are largely, at least, of foliar origin.

Stanford University

THE RELATION OF PLANT PATHOLOGY TO HUMAN WELFARE[1]

F. L. Stevens

(Received for publication January 17, 1921)

In the present era of high cost it is especially fitting that one take account of all expenditures, and weigh carefully the returns. With the present underpaid and poorly equipped condition of many educational and research institutions, and especially in the light of certain criticisms that are made regarding research, I am impelled today to select from the many interesting themes which might be developed regarding plant pathology, and to direct thought to research in the *science* of plant pathology and its related fields, and briefly to indicate the returns therefrom.

Plant pathology is preeminently a practical science, and its prime function is to guide the way to an ever-increasing control over disease.

The magnitude of the annual loss incurred in the United States alone through plant disease in diminution of yield and loss of produce is far greater than it is generally conceived to be. I shall not burden you with statistics, but I do wish to give a few examples, taken from the most reliable estimates that have been made, to indicate the loss. Thus, in the various Plant Disease Survey Reports we find that for the year 1919 losses from plant diseases are given as follows: For the five leading cereals 482,695,000 bushels; for potatoes 86,997,000 bushels; for tomatoes 307,168,000 bushels; for sweet potatoes the loss is put at 58,841,000 bushels, or more than one half the crop. You are all familiar with the diseases mentioned; but you fail to get the world bearing of plant-disease ravages unless you include in your vision such destructive diseases as the coffee rust, affecting in disastrous form a crop of large world value, which in two years destroyed 272,000 acres in Ceylon; the banana wilt, which is reported to have caused abandonment of nearly 20,000 acres of banana plantings in Panama alone and to have rendered useless large railroad lines; the cocoanut palm bud-rot, which kills the growing point of this valuable tree and which is rapidly encircling the world.

Your imagination may fairly picture similar diseases as occurring throughout the world on the whole range of useful plants. Before harvest disease may devastate the crop in the field, and after harvest the inroads proceed in storage. Obviously the loss occasioned by destruction of the product at market is far greater per unit than similar losses in the field.

---

[1] Invitation paper read before the joint session of Section G, A.A.A.S., the Botanical Society of America, and the American Phytopathological Society, in the symposium on "The Relation of Botany to Human Welfare," at Chicago, December 29, 1920.

Thus a carload of Georgia peaches spoiled by brown-rot in New York means loss of transportation and handling as well as of the original value of the fruit. An annual sum of $30,000,000 is said to be a conservative estimate of the loss in the United States between the field and the consumer, while in 1919 the total loss with fifteen principal food products is estimated at nearly a billion and a half dollars. Even land values are frequently seriously depreciated when the soil is so infested as to preclude the raising of the particularly profitable crop, as, for example, when the tobacco wilt possesses land in the bright tobacco belt, leaving a farm which is comparatively worthless for any other crop, or again, as I have seen, when the wilts of cotton, cowpeas, and melons all occur upon the same field.

As civilization advances, intercourse between regions more or less remote increases and the disease range and prevalence expand. Thus, much as with human and cattle diseases, though to much greater extent, the number of plant diseases known in any community is annually increased by additions from near-by regions or from far-away continents. Presumably the potato late-blight fungus began its journey of conquest in the Andes, and as early as 1845 caused famine in Europe and much loss in many continents. The asparagus rust appeared in New Jersey in 1896 and spread until it reached California in 1901. Many other serious diseases have come to us from abroad, including the sorghum smut, grape anthracnose, cucurbit mildew, carnation and chrysanthemum rust.

Numerous serious diseases have likewise invaded other countries from here, among them the grape black-rot and downy mildew. Of interstate migration interesting cases are afforded by pear blight, from the Hudson valley in 1792 to California in 1895, and by peach yellows from Philadelphia in 1806 to Maine and Illinois in 1886. Among the late continental arrivals are the pine blister rust, which is under such headway that it seems to be impossible of extermination. The value of the susceptible pines is such that the loss may readily reach a hundred million dollars.

The chestnut-bark disease caused a loss of $25,000,000 from 1904 to 1911. Much more serious is the loss to be borne as it invades the great chestnut forests of the Appalachians. Citrus canker, imported from Japan about 1910–11, bids fair to ruin large industries. Potato wart entered Newfoundland in 1909 and was found in Pennsylvania in 1918. It is of interest to note in passing that, were agriculture not taught in the public schools, its presence might yet be unsuspected. Flag smut of wheat was undiscovered in America until May 5, 1919, and is as yet known in but one county in Illinois. This disease is said to cause loss ranging from 10 to 50 percent in Australia.

As increased long-distance communication gives intercontinental transport to disease, so congestion of crop population creates a bridge by which the causal organism may more readily pass from plant to plant or from farm to farm. In these two conditions, facility of transportation and congestion of crop, we find, to a large degree, explanation of the fact that plant diseases are more prevalent now than formerly.

The multiplicity and diversity of plant diseases are especially striking. While the physician has but one species of patient and the veterinarian but a few species, the phytopathologist has to advise regarding many species of plants each of which has, to a great extent, its own large list of diseases. Thus, on the apple alone there are 18 major diseases; on wheat 10; on potatoes 12; while for each crop the number of minor diseases is more than ten times as great.

I have attempted thus briefly to indicate the damage done by plant disease, as a background for a discussion of the part played by plant pathology. The point of importance is not how great is the loss from plant disease, but rather how much influence has the science of plant pathology had in lessening this loss.

Like bacteriology the science is young, dating back barely to the middle of the last century. It was first taught in any American college in 1873 (Illinois), and first as a special subject in 1875 (Harvard). The science has grown until today the American Phytopathological Society enrolls nearly 500 members, the majority of whom are professional plant pathologists, and whereas but one paper appeared in America on the subject in 1861, each month now adds scores of titles and nearly a hundred papers are presented here this week. Large federal and state appropriations sustain its researches.

What is the nature of the return that plant pathology has given? The achievements may be summarized briefly as falling within seven great categories demonstrating the value of: protective applications, sprays and dusts; excision; seed steeps; general sanitation leading to diminution of infective material; breeding for disease resistance; modifications of agricultural practice; quarantine restrictions.

It is unnecessary to discuss these, but I wish to point out that while a modicum of the present benefit doubtless would have obtained from an empirical, rule-of-thumb procedure, the great body of our present knowledge of disease prevention is the direct outcome of truly scientific investigation. It is difficult as you journey from coast to coast today, and see spraying practiced everywhere, to realize that prior to 1885 no spraying was done in the United States. The vast sums spent for copper sulphate, lime-sulphur, etc., and the large factories devoted to making spraying machinery also attest the wonderful growth of this custom. Yet it was not the accident associated with the stealing of wayside grapes that was responsible for the discovery of the efficiency of fungicidal applications; it was the close observation of Millardet followed by his keen analysis and exact experimentation, all of which would probably have failed were it not for the basic knowledge that Millardet had regarding fungi and parasitism. His receptivity of mind was doubtless dependent upon mycological studies of many decades.

Heteroecism of apple and wheat rust and hibernation of many fruit-rot fungi in cankers or mummied fruits, which in the light of science are simple, easily comprehended facts, could without science have had but little more than the force of superstitions. The investigations which have given greatest value to seed steeps have been those that showed the part played by seedling and floral infection. Recommendations of general sanitation would be largely without force were it not that the underlying reasons were made obvious by scientific explanation. Of all the categories mentioned, perhaps the least dependent upon science and the most empirical is that relative to disease resistance, since some of our most valuable resistant varieties have been given to us by farmers, while many of the most susceptible have been eliminated naturally. During recent years, however, knowledge of Mendelism and of biologic specialization has added a very important, truly scientific aspect to this somewhat empirical subject.

Many crops are of such small acreage value that expensive methods of disease prevention permissible with more valuable crops are precluded. In such cases, modification of practice, as change of time of seeding, of crop rotation, of kind of fertilization, of degree of drainage, of age of seed, of depth of plowing, of proper relation of direction of rows to wind and light, has in many cases proved serviceable. The suggestion of such modifications depends upon most intimate knowledge of both crop and parasite, and full life-history studies of the ecology of the organisms are needed. It is obvious that for the establishment of proper quarantine restrictions the taxonomy and morphology of the causal organisms must be known.

It is both impossible and unnecessary to assign any money values to the protection that has been given to American crop plants under the various categories mentioned. A few cases illustrative of efficiency may, however, be mentioned. Cereal seed steeps at a cost of less than three cents an acre effect practically complete elimination of certain smuts. Thus the saving of oats in one state with full utilization of this knowledge would be about 7,000,000 bushels. One spraying for peach curl is stated to prevent 98 percent of the injury with a net profit of more than $400 an acre. Innumerable other examples over the whole range of crop production could be adduced. Perhaps the most striking cases of value of our science occur in connection with quarantine restriction and early extermination of an invading disease. Coffee rust reached Porto Rico in 1902 on stock brought to Porto Rico by a Dutch battleship from the East Indies. It was early recognized by the experts of the Agricultural Experiment Station at Mayaguez, and, though a foothold had been gained, the disease was exterminated. So complete was the elimination that not even a herbarium specimen of the rust can now be found in Porto Rico. If you will visualize the coffee plantations of America paralyzed by this devastating disease, you will give due thanks over your morning coffee to the efficiency of the Porto Rican Station's activity. Another notable eradication was that of the rice smut, properly named *Tilletia horrida* by the Japanese, which was eradicated from South Carolina in 1898 by Dr. A. P. Anderson.

Citrus canker was more tardily recognized, but the expenditure of $1,500,000 by Florida to protect a crop worth $50,000,000 annually and promising to be worth twice that is freely made. The number of cankered trees found in Florida in 1915 was 6,715. In 1919 it was reduced to 4. Perhaps the most significant of cases is that of flag smut of wheat. Picture this as spread over the wheat areas of the United States with an annual loss of nine million bushels to occur over a long period of years. Let us hope that the gravity of the situation is realized and that the appropriation and activities suffice not only to hold it within its present limited range of one county, but actually to eliminate it.

The decay of structural timber, while not, strictly speaking, due to disease, falls within the province of pathology. I can merely hint at the benefits that occur through activities in this field. Certain plant diseases, as the ergots of grain and grasses, have caused serious inroads upon human health and that of cattle. These the science of plant pathology alleviates. I trust that I have given you a partial picture, a mere glimpse here and there, to indicate the manifold, broad, important relations existing between the science of phytopathology and human welfare. Such in general are the field and the achievements, the relations of the science. All the facts that I have presented were doubtless known to many of you; perhaps some were not known to all.

The utility of the science is broadly attested and indeed is unquestioned. Benefits almost inconceivable will result from such extension, or other propaganda, as bring into actual use the knowledge that science has already given us. With the further accumulation of knowledge by the present types of research, other vast benefits will arise regarding each one of the numerous diseases. None is so well studied that further searching will not be rewarded, as is attested by many recent investigations. Accurate knowledge of the flight of sporidia of Gymnosporangium or of ascospores of Venturia, for example, may lead to important modifications of practice. Bud hibernation of the mycelium of a previously much studied group of fungi was but recently discovered.

It is to be observed that the great discovery of the parasitism of the fungi and the founding of bacteriology and the development of its methodology, together with the early foundations laid through the years in histology, mycology, taxonomy, and physiology, have furnished the bases on which plant pathology has made its advance. Aside from these there have been few, if any, great fundamental contributions.

In the earlier days, descriptions, recognition of causes, and trials of obvious prophylactic measures was the usual order. Officials and the public desired immediate recommendations. This type of work as regards the really important diseases has largely been done, and now is the period of more complete, exact study, to lead to new knowledge of fundamental utility. There still remain many problems concerning each disease and many concerning disease in general, but they are for the most part deep and fundamental, not superficial.

Pathology has used cytology to determine relationships and clarify understanding, histology to aid in the interpretation of the morbid, and is constantly dependent upon taxonomy and physiology. Yet, owing to an organization demanding direct application and lacking in opportunity of specialization, and conducive to dissipation of energies over many really distinct fields, these fundamental branches of our science fail to keep abreast of the needs. Taxonomy of the parasites, never satisfactory, in the light of recent discoveries regarding biologic specialization and heredity, is much less so. Few of the many large genera of imperfect fungi, as Phyllosticta, Septoria, Cercospora, have been studied even from a morphological viewpoint. Their cytology, enzymology, life histories, ecology, variability, genetics, are almost unknown. The studies made with a few genera offer suggestions as to what may be done morphologically in such fields, while studies in biologic specialization with the rusts and powdery mildews indicate the need of similar studies with the fungi imperfecti.

The problems of disease resistance and wherein it lies are obviously important. Why, for example, may pear blight proceed at the rate of several centimeters a day down a twig, then suddenly cease to proceed further? Why are some Alternarias parasitic, others not? Questions of inheritance of disease resistance are very complex, and much fundamental work of high value needs to be done. Enzymes and toxins will repay much study. That group of mysterious diseases including the mosaics and peach yellows holds a secret the discovery of which may well be revolutionary in pathology.

Had I time or you patience, scores of problems of equal importance could be mentioned. Many great problems exist, and that they will slowly give way to patient, scholarly attack may with confidence be expected. But since the problems now before us are more intricate than those of the past generation, they demand concentration, larger breadth of equipment, longer periods of sustained research on a given problem, in a word, greater specialization, and this often needs be accompanied by cooperation of widely separated branches of science or of distinct sciences.

The pressure for immediate results, well enough in the era now closing, and which has served its purpose in demonstrating to the world the value of knowledge of pathology, is not the only force that should impel the pathologist to further work. A great part of all research in pathology is now fostered by either federal or state aid, yet Sir A. D. Hall, chief scientific advisor to the Ministry of Agriculture of Great Britain, says that "a government is unfitted by its very nature to conduct fundamental research," and adds that "the scientific man in self-protection . . . is tempted to take a short point of view and not only to do work which will give immediate results but to produce these results very early. Awful examples can be quoted." He says also that "under compulsion of justification there is much danger that the rest of the work is forced to conform to the initial misconception." Without in any way belittling the progress that is being made through the large volume of work of high quality that is done under the present régime, I may add that similar quotations are available from American writings.

Pathology is now become of such large scope that it is beginning to differentiate. It is now demanded that common diseases be diagnosed and treatments recommended, extension work done, surveys made, fungicides and spraying schedules tested, quarantine restrictions enforced, seeds certified as free of diseases, and similar other time-consuming duties attended to. Thus should, and does, arise a division of labor, giving the administration of these important fields to those adapted to them by ability and inclination and leaving the pathologist more nearly free to make his laboratory and field attack upon other problems. This freedom is already reacting favorably upon research in pathology and altering perceptibility the character of the output. But it is clearly apparent that the existing agencies and the motives engendered by pressure of present conditions will not lead to that full, broad, fundamental development that is needed.

More adequate study of the fundamentals clearly means that pathology must come into closer coöperation with all other phases of botany and indeed with other sciences. Such coöperation that mycologists, bacteriologists, physiologists, taxonomists, technicians, organic, physiological, and physical chemists, may together concentrate upon some worthy problem, such, for example, as disease resistance, or the mosaics. This coöperation can perhaps best be brought about by the establishment of an institute, in such manner as to secure not only the desired coöperation but the freedom from time pressure that is needed.

The very success of pathology is in itself a danger in that numerous positions successfully tempt the student to leave his training only partially completed, and, moreover, deplete the ranks of fundamental botany, taking from them those whose abilities and inclinations would otherwise assure their aid in fields kindred to pathology.

A second movement that would tend much to relieve the deficiency would be one that would encourage the individual worker, accentuating a revival of that spirit which impelled Berkeley, the Tulasnes, de Bary, Brefeld, Farlow. It is in the universities and colleges that their successors will largely do their work. Our indebtedness to such isolated workers in the past is clearly recognized, and without their aid in the future we lose much. The necessary limitations of a project system would have precluded the great discoveries of the past, the results of the genius of Darwin and Pasteur, as, indeed, under the project system it is detailed rather than fundamental contributions that are made. I may summarize these points as follows: That, notwithstanding the truly enormous output of high quality and great utility from government institutions, the limitations inherent in their organization are such that certain important deficiencies are apparent. That an institute or institutes for pathology, properly organized, would partially meet these deficiencies. But that, as in the past, we must still look to the isolated students, fired with enthusiasm and willing to devote a life-time to the development of their fields to buttress the structure of pathology and add to its foundations.

Chemistry as a science is strong in numbers and influence because she has maintained her unity. Botany is less a power than she should be because each useful offspring has gone regretfully far from the region of maternal influence. Witness the present separateness of bacteriology, of botany as applied to agriculture, forestry, horticulture, and to an extent to plant pathology. Integration of the various phases of botany rather than further disintegration is to be desired.

Harvard Summer School

JULY 5 to AUGUST 13

1921

Courses in Elementary Botany, Morphology, and Plant Anatomy are offered by the Harvard Summer School for 1921. A limited number of research students can be accommodated in Botany S20a. Correspondence in regard to the various courses should be directed to Professor E. C. JEFFREY, 47 Lakeview Avenue, Cambridge, Mass., until the 5th of June. From that date until the opening of the School, inquiries should be addressed to Dr. R. E. TORREY, Department of Botany, Massachusetts Agricultural College, Amherst, Mass. Requests for information may also be sent to the Director of the Harvard Summer School, 19 University Hall, Cambridge, Mass.

UNIVERSITY OF COLORADO

SUMMER QUARTER

Location: Boulder, Colorado, at the mountain front of the Rockies; environment favorable for study, for field work, and for outdoor recreation. Easy access to all of the life zones from plains to alpine heights and glaciers.

Courses in Botany and Zoology: College Botany, Economic Botany, Bacteriology, Botany of Ornamental Plants, Ecology, Local Flora, Forest Botany, College Zoology, Nature Study, Ornithology, Economic Zoology, Cytology, Heredity.

Other Courses in all usual academic subjects.

Summer quarter of two terms: June 13 to July 21 and July 21 to August 27.

Catalogue may be obtained by addressing the Registrar of the University, Boulder, Colorado. For any special information regarding courses in botany and zoology address Professor Francis Ramaley, University of Colorado, Boulder, Colo.

Our Professional Advancement Service

Produces Results

A recent notice brought fourteen replies in four weeks

For terms see elsewhere in this issue

Address

American Journal of Botany
Brooklyn Botanic Garden
Brooklyn, N. Y.

ECOLOGY

Devoted to all phases of Ecological Biology

Official Publication of the

Ecological Society of America

Quarterly

Current, $3.00 a Volume
Back volumes, $3.50

Sample Copy on Request

Address

ECOLOGY
Brooklyn Botanic Garden
Brooklyn, N. Y., U.S.A.

Had You Ever Thought This About The Greenhouses We Build

Had you ever thought that if we didn't use quite such ornamental hoods over the doors; and made the eaves straight instead of curved, that although the house might not be quite as attractive it would be just as good a growing house and exactly as durable?

In other words, the kind of upholstery in a car has no effect on the hill-climbing power of the engine.

To all of which we heartily agree.

Furthermore, we build just such a house as you describe.

It's called our Practical Purpose house.

Glad to give you figures just to show you how decidedly practical it is when it comes to the price.

Hitchings and Company

Home Office and Factory: ELIZABETH, N. J.

NEW YORK
101 Park Ave.

BOSTON-9
294 Washington St.

Vol. VIII. JULY, 1921 No. 7

AMERICAN JOURNAL OF BOTANY

OFFICIAL PUBLICATION OF THE
BOTANICAL SOCIETY OF AMERICA

Smithsonian Institution
SEP -3 1921
National Museum

CONTENTS

The relation of crop-plant botany to human welfare . . . CARLETON R. BALL 323
Correlations between anatomical characters in the seedling of Phaseolus vulgaris.
J. ARTHUR HARRIS, EDMUND W. SINNOTT, JOHN Y. PENNYPACKER and G. B. DURHAM 339
A quantitative study of the effect of anions on the permeability of plant cells—II . . . . ORAN L. RABER 366
The mechanism of root pressure and its relation to sap flow
JAMES BERTRAM OVERTON 369

PUBLISHED
IN COOPERATION WITH THE BOTANICAL SOCIETY OF AMERICA
BY THE
BROOKLYN BOTANIC GARDEN
AT 41 NORTH QUEEN STREET, LANCASTER, PA.

Entered as second-class matter February 21, 1914 at the post office at Lancaster, Pennsylvania, under the act of March 3, 1879

AMERICAN
JOURNAL OF BOTANY

DEVOTED TO ALL BRANCHES OF BOTANICAL SCIENCE

ESTABLISHED 1914

EDITED BY A COMMITTEE OF THE
BOTANICAL SOCIETY OF AMERICA

EDITORIAL COMMITTEE
C. E. ALLEN, Editor-in-Chief,
University of Wisconsin

WILLIAM CROCKER,
University of Chicago

C. STUART GAGER, Business Manager
Brooklyn Botanic Garden

R. A. HARPER,
Columbia University

L. R. JONES,
University of Wisconsin

ORLAND E. WHITE,
Brooklyn Botanic Garden

JACOB R. SCHRAMM,
Cornell University

C. L. SHEAR, *Bureau of Plant Industry*
(Representing American Phytopathological Society)

The Journal is published monthly, except during August and September. Subscription price, $6.00 a year. Single copies 75 cents. Back numbers, 75 cents each; $7.00 a volume. Postage will be charged to all foreign countries, except Mexico, Cuba, Porto Rico, Panama Canal Zone, Republic of Panama, Hawaii, Philippine Islands, Guam, Samoan Islands, and Shanghai. Postage to Canada, 20 cents a volume on annual subscriptions; to all other countries in the Postal Union, 40 cents a volume on annual subscriptions.

The pages of the Journal are open to members of the Botanical Society of America, or to candidates approved for membership.

Manuscript offered for publication should be typewritten, and should in all cases be submitted to the Editor-in-Chief.

Papers are limited to 20 pages in length, exclusive of plates, except that additional pages may be arranged for at cost rate to authors (approximately $3.00 a page).

Proofs should be corrected immediately on receipt, and returned to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y.

Illustrations, other than zinc etchings, and tabular matter should, in general, be restricted to approximately ten percent of the number of pages of text. Authors may be charged at cost rates for illustrations and tabular matter in excess of that amount (approximately $6.40 a page for tabular matter; 15 cents per square inch for half-tones).

Separates should be ordered when proof is returned. Fty copies without cover will be supplied free; cover and additional copies at cost.

Remittances should be made payable to American Journal of Botany. Ten cents must be added to all checks not drawn on New York City banks.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

Correspondence concerning editorial matters should be addressed to Prof. C. E. Allen, University of Wisconsin, Madison, Wisconsin.

Business correspondence. including notice of change of address, and directions concerning reprints, should be addressed to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y., or 41 North Queen Street, Lancaster, Pa.

Smithsonian Institution
SEP - 8 1921
National Museum

AMERICAN
JOURNAL OF BOTANY

VOL. VIII JULY, 1921 No. 7

THE RELATION OF CROP-PLANT BOTANY TO HUMAN WELFARE[1]

CARLETON R. BALL

(Received for publication January 17, 1921)

It is with combined trepidation and pleasure that I endeavor to discuss before this gathering the subject of the relation of the botany of crop plants to human welfare. Trepidation because of the difficulty of adequately presenting so important a subject. Pleasure because I believe that a vital relation exists between the botany of crop plants and human welfare and rejoice at the opportunity to emphasize it to others. Let us define what we shall discuss together.

BOTANY

What is botany? The dictionary tells us that it is the science of plant form, structure, function, relationship, and distribution. From this category we evolve several subdivisions of the science; for example:

Phytomorphology, the science of plant form and structure;
Phytophysiology, the science of plant function and growth;
Phyto-ecology, the science of plant response to environment; and
Phytotaxonomy, the science of plant relationships.

Some of these major divisions are themselves subdivided. For instance, taxonomy includes phytography, or plant description; taxonomy proper, or plant classification; and nomenclature, or plant naming, the black sheep of the family. In like manner, plant ecology includes plant physiology and phytogeography, or plant distribution.

In addition to these grand divisions of botany there are some important specialized phases of botanical science, such as phytopathology, or plant diseases; pharmacognosy, or pharmaceutical botany, dealing with medicinal plants; and phytopaleontology, or paleobotany, the science of fossil plants, sometimes called fossil botany. Phytopathology, in turn, includes mycology, or the taxonomy of fungi; physiology, or the function of both host and parasite; and ecology, or environmental response. Finally, there is

---

[1] Invitation paper read before the joint session of Section G, A.A.A.S., the Botanical Society of America, and the American Phytopathological Society, in the symposium on "The Relation of Botany to Human Welfare," at Chicago, December 29, 1920.

[The **Journal** for June (8: 275–322) was issued June 30, 1921]

genetics, the science of the origin and expression of characters, the sum total of which makes up the organism as we know it.

Because of the symposium arrangement, it is necessary to restrict the treatment of the theme so as not to trench on the subjects assigned to my colleagues, Dr. Cowles and Dr. Stevens. Omitting their topics, the relation of ecology and of pathology, respectively, to human welfare, there remain the morphology, physiology (aside from environment), and genetics of crop plants, as well as pharmacology or pharmaceutical botany.

At the start we are confronted by the well-known dictum of one class of botanists that, whenever botany relates in any way to human welfare, by that very fact it ceases to be botany. One is reminded of the famous couplet written in similar vein:

> My name is Benjamin Jowett,
>   Master of Balliol College,
> Whatever is known, I know it,
>   Whatever I don't, isn't knowledge.

It may be argued that this dictum is but a theory, a state of mind. To those who cherish that delusion it should be necessary only to point out a few striking facts of the botanical past and present.

Development of Botanical Science

Until comparatively recent years practically all botanists studied wild and domesticated plants impartially. This was true of the Greek philosopher-naturalist, Theophrastus, of the third century B.C., the father of modern descriptive botany. It was true of the Greek physician, Dioscorides, and of the Roman, Varro, in the first century B.C., and of the Roman essayist, Pliny, in the first century of the present era. It remained true of botanists in general until toward the middle of the nineteenth century, or some 75 to 100 years ago.

With the revival of learning after the Dark Ages came a renewed expression of interest in things botanical. The first important books of a distinctively botanical character were the so-called herbals, running from those of Ruel in 1537 and Fuchs in 1542 to those of Ray and Morison in 1688 and 1699, respectively. These ponderous folio volumes, written in quaint and not always accurate Latin or in no less quaint English, and illustrated with crude but often startlingly realistic woodcuts, served as repositories of popular and semi-scientific information on plants during the sixteenth and seventeenth centuries. They were based on the writings of the Greek and Roman authors named above, but contained many original observations. Many of the plants treated by the herbalists were in common cultivation, and in that period the crop plants received the same botanical attention as did the feral species. Thus we see that the important early contributions to botanical science were in the field of what, in recent years, unfortunately has been called *applied* or *agricultural* botany.

Throughout the seventeenth century, botanical knowledge was growing rapidly along taxonomic lines. Master minds were laboring to formulate orderly classifications of both wild and domesticated plants. The later and more logical of these attempts are typified in the writings of Tournefort and the elder Jussieu and reached their climax in the eighteenth century in the classic works of Linné, in 1737 and 1753, and in those of the contemporaneous Jussieu the younger. By these and succeeding systematists of the eighteenth century and the first half of the nineteenth century, the leading species and subspecies of important cultivated plants were included and given specific rank as readily as if native and wild.

The writer does not believe that the feral and cultivated species should have received identical treatment, for differences in morphological characters sufficient to separate species of wild plants are sufficient only to separate agronomic or horticultural varieties of crop plants. The point to be emphasized is that the cultivated plants were felt to be worthy the attention of the greatest botanists of all those centuries.

Before the middle of the nineteenth century, a change of attitude had become fully evident. Discussion of crop plants was eliminated from manuals of botany. This change of attitude probably was due to several different reasons. The first, doubtless, was a realization of the difficulties of classifying cultivated crops on the same basis as wild plants. The second was the marvelous improvement in methods of transportation and communication, through the development of railroads and postal facilities. These made possible the botanical exploration of the hinterlands and distributed the resulting collections to botanists everywhere for study, thus diverting attention from domesticated plants. The third and perhaps controlling reason was a growing feeling that useful plants were of a class apart, botanically unclean, and unworthy the best thought of the systematist and physiologist. The result was that, while a few botanists devoted themselves almost exclusively to studies of crop plants, the great majority shunned them entirely. That such a situation should have developed at all was most unfortunate, but that it should have come about just in that period of time was particularly deplorable.

The present era of widespread appreciation and subsidization of the biological sciences was about to be ushered in. The act establishing the system of land-grant colleges was in process of formation. The founding of the state agricultural experiment stations was only 30 or 40 years ahead. The increased appropriations for research in nation and states were to follow soon after. The amazing endowment of great universities was on the horizon. The organization of such great scientific bodies as those in session here was about to become an accomplished fact.

One can almost imagine that the scientific sky must have been aglow with the coming dawn. In spite of this, Botany drew apart and proclaimed herself too sacred to be polluted by the useful. The pursuit of truth for truth's sake is noble, and consecrated thousands have devoted themselves to lives of privation and sacrifice under the inspiration of this ideal. Truth for man's sake is no less noble. If in social science, service to others is the highest form of altruism, how unreasonable the attitude that, in the development of a natural science, no thought for humanity should be allowed to enter.

From our present vantage point of perspective, it seems doubly unfortunate that the botanical fraternity should have lost interest in domesticated plants just at a period when the development of teaching and research institutions would have given them the needed laboratory and field facilities for really effective study. Farm crops as a subject was not and is not taught by botanists. As the writer has pointed out in a previous paper, the original farm-crop specialists entered that field through many doors, including chemistry, the old-time agriculture, and even animal husbandry, as well as botany. On the other hand, when botany did deal with any phase of farm crops she called it "applied botany" or "economic botany," and so erected the wall which gradually shut her off from part of her own domain. From this illogical and unfortunate separation, both botany and agronomy suffer to this day.

Human Welfare

Human welfare may be defined as a satisfactory condition or relation of human society, individually and in the mass. Such welfare must be both material and esthetic. It presumes a coördination of good in the physical, mental, and spiritual realms. Can the botany of crop plants be shown to have any relation to these phases of human welfare? Let us ascertain.

Upon the products of the vegetable kingdom the human race depends for the very essentials of its life. The vital needs of humans are twofold, food and shelter, and these are important in the order named. The great classes of useful plants which minister to these two needs are cereals, fruits, vegetables, forages, saccharines, and medicinals among the foods, and fibers and forest supplies among the materials providing shelter.

Human Food

Human food is chiefly either animal or vegetable in origin. Primitive man doubtless was both herbivorous and carnivorous. Wild animals furnish as abundant and palatable a food supply as do domesticated animals, but the same is not true regarding feral and cultivated plants. It seems probable, therefore, that primitive man used meat as his staple food, and used vegetable materials to maintain health, to vary his diet, or as a filler. In temperate areas, at least, the necessary supply of roots, fruits, and seeds was obtained only by arduous search and tedious labor in gathering. It could have been abundantly obtainable, also, only during a portion of the year, which would have been less true of animal food supplies. On the other hand, vegetable food materials were more readily preserved for future use than were meats.

The story of the first attempts at cultivation of food crops is lost in the mists of prehistoric time. Seeds dropped about a favorite camping site by members of a nomadic tribe, during preparation or eating, may have produced plants after the family moved on. Later, when they passed that way again, the mature plants with ripened seeds or fruits may have attracted their attention as providing a convenient food supply in a concentrated area. From some such chance observation may have developed the rudiment of the idea of growing the food plant where it was to be used. And no true welfare of human society was possible until man could produce and store food supplies against a time of scarcity and need.

We can even imagine that the quantity and nature of the food supply had its influence on developing mentality. Perhaps there arose, even in those days, the superman who ruled by brain as well as by brawn. And perhaps also there was not wanting an envious Cassius to exclaim: "On what meat doth this our Caesar feed, that he is grown so great?"

The Future Food Supply

The chief problem of the world in the immediate future is the food supply. Across the pages of history one clear record runs. That nation is most secure which has, or can insure, adequate resources of food. Napoleon said that an army travels on its stomach. Not only of the army but of the nation itself is this true. Two thousand years ago the grain ships from Egypt sailed the Mediterranean to imperial Rome. Today the grain ships ply the seven seas to imperial Britain. They go from Australia and Argentina, from India and from Canada, and even from the United States itself. Tomorrow they may be steaming toward our shores, carrying a similar cargo.

Ever since American agriculture advanced from the forest clearing to the open prairies and the boundless plains, our country has been a heavy exporter of foodstuffs. Not once have we had to stop and consider from across what seas grain ships should come to us. But now the old order changeth and giveth place to new. With a population increasing rapidly through birth and immigration, and with no large areas of cheap and fertile lands remaining to be brought under cultivation, we come face to face with the problem of our future food supply. Our exports of vegetable foodstuffs are steadily declining as more and more is required at home. Our imports of food materials are steadily mounting as the need increases.

This is not written as a pessimistic prophecy but as a sane realization of an imminent problem in order that an adequate solution may be sought. The solution lies in one or more of three directions. First, immediate restriction of immigration and finally the restriction of the birth rate through economic pressure. There are some well recognized but unfortunate biological facts which make this undesirable. Second, an endeavor to import what we do not produce. This puts us at the mercy of other nations in ways of which we have had recent illuminating examples. Third, an undertaking to increase our own production of food to keep pace with increase of population. This last is the only satisfactory decision. How may this result be accomplished?

There are two chief lines of attack on the problem of increasing our food supply. One is to increase the area under cultivation, by reclaiming desert areas through the more extensive and more productive use of irrigation waters, by the reclamation of swamp lands, and by the utilization of the untilled lands in the present tilled area. Some of these are problems in engineering, some in economics, some in soil science, and some in crop physiology. The other possibility is to increase the productivity of the areas already farmed. This requires progress in farm organization, soil science, animal husbandry, crop rotations, plant improvement, plant introduction, and the control of crop pests. Both methods present plant problems which challenge botany to her utmost endeavor.

Botanic Families of Important Crop Plants

We have seen that the crop plants vital to human welfare are those which furnish food, fodder, and medicine for man and his domesticated animals, clothing for him, and shelter for him and for them, and also for his industries. Plants from almost the entire range of the vegetable kingdom are requisitioned to provide material for one or another of these purposes. This realization recalls the promise of Scripture:

> And God said, " Behold, I have given you every herb yielding seed, which is upon the face of all the earth, and every tree which is the fruit of a tree yielding seed, to you it shall be for food " (Genesis 1: 29, Am. Rev.).

The three important classes of food plants for man are the cereals, the vegetables, and the fruits.

Cereals

No other botanic family is of such overwhelming significance to the human race as the grass family, Poaceae or Gramineae, which contains the cereals, corn, wheat, rye, oat, barley, rice, sorghum, and millet, as well as the most important hay, grazing, and silage crops. For those who like statistics, it may be of interest to note that the estimated value of the cereals grown in the United States in 1917 was over $6,800,000,000; in 1918 about the same; and in 1919, nearly $7,300,000,000. Out of sympathy for any botanists so unlucky as to own grain farms, the figures for 1920 are omitted.

A few other plants ordinarily are classed as cereals, though not truly such. Among these is buckwheat, belonging to so distant and unpromising a botanic family as the Polygonaceae, while the closely related family, Chenopodiaceae, contains quinua, a human food extensively used by the primitive Andean tribes. Other plants which are cereal substitutes, in that they furnish starch in concentrated form, such as the potato and similar plants, are discussed under vegetables.

Vegetables

It is somewhat surprising to note how few plant families contain the great majority of the common vegetables of the temperate world. From the Solanaceae come the potato, tomato, and eggplant, not to mention cayenne pepper, the ground cherry, and tobacco, the petunia and the matrimony vine. The Leguminosae furnish beans, peas, lentils, cow peas, soy beans, and peanuts. Another family furnishing a large number of edible roots and plants is the Cruciferae, or mustard family, containing the radish, turnip, and rutabaga, the cabbage and its congeners and derivatives, and the horseradish, cress, and mustard, not to mention such flowers as candytuft, sweet alyssum, wall-flowers, rockets, and gillyflower. From the sunflower family, Compositae in the broad sense, we get lettuce, salsify, chicory, endive, sunflower, and artichoke. The melon family, Cucurbitaceae, contains many large and striking products, as pumpkins, squashes, cucumbers, gourds, gherkins, cantaloupes, casabas, watermelons, and citrons.

In addition to the five large families listed above, are several with fewer economic species. Carrot, celery, parsley, and parsnip represent the Umbelliferae; rhubarb the Polygonaceae, and beets and spinach the Chenopodiaceae. Asparagus and the various onions represent the Liliaceae, while the sweet potato is a morning glory, belonging to the Convolvulaceae, the taro and dasheen belong to the Araceae, and okra belongs to the Malvaceae, with cotton.

Finally, the puffballs, mushrooms, and truffles are fungi, belonging to different families of that large group called Basidiomycetes.

Fruits

The family Rosaceae, used in the inclusive sense, probably furnishes more of the fruits grown in the temperate zone than all other families combined. Among its members are the apple, pear, quince, peach, apricot, plum, cherry, blackberry, raspberry, strawberry, juneberry, and almond, not to mention roses, spireas, and other flowers. Closely related is the family Grossulariaceae, containing the currants and gooseberries.

The citrus family, Rutaceae, ranks next to the Rosaceae in the number and importance of its products, which include the orange, lemon, grapefruit, citron, lime, tangerine, and others. The family Vitaceae probably stands third in rank, with its numerous and varied kinds of grapes, including the so-called currant of commerce.

Other important fruits are the date and coconut, of the Palmaceae, the banana, of the Musaceae, the olive, of the Oleaceae, and the pineapple, belonging to the Bromeliaceae, which includes also the so-called Spanish moss of our southern forests. Such fruits as the blueberries and huckleberries (Vacciniaceae), the persimmons (Ebenaceae), the papaw (Anonaceae), and the mulberry, fig, and breadfruit (Moraceae) should not be forgotten.

Nor should the importance of nuts be overlooked. In nut production, the family Juglandaceae takes first rank, with its walnuts, butternuts, hickory nuts, and pecans. The family next in importance is the Fagaceae, containing the chestnuts, the beechnuts, and the numerous acorns, so important as foods for primitive peoples as well as for animals. Of third rank, probably, is the family Palmaceae, if the widely distributed and important coconut is counted a nut rather than a fruit.

Forages

Among forages the grass family, Poaceae, has by far the largest number of representatives. Chief among the cultivated grasses are timothy, bluegrass, redtop, orchard grass, meadow fescue, bermuda, and sudan grass, as also the cereals. A multitude of native species furnish grazing. Next in importance stand the legumes, Leguminosae, of which alfalfa, clovers, sweet clovers, vetches, cow peas, soy beans, velvet beans, and peanuts are well known and valuable representatives. Beyond these two great forage families stretches a long line of other families some of whose members are grazed, browsed, or ensiled, or otherwise enter into the animal diet.

Saccharines

The principal saccharines are sugar cane and sorgo, both grasses; the sugar beet, like the garden beet, of the Chenopodiaceae; and the sugar maple, belonging to the Aceraceae.

Medicinal and Poisonous Plants

Even to mention the many natural families yielding healing and toxic substances is beyond the scope of the present paper. Certain important examples will occur readily to all. Probably the three most important drug-producing families are the Papaveraceae, or poppy family, yielding opium and its derivatives, so useful in relieving pain, but so terrible in their effects when abused; the Rubiaceae, or madder family, producing the quinine so potent in the control of malarial fevers, as well as coffee; and the Solanaceae, or potato family, producing belladonna, capsicum, stramonium, and tobacco. Other prominent drugs are found in the Araceae, Compositae, Cruciferae, Labiateae, Leguminosae, Ranunculaceae, Rosaceae, and Umbelliferae.

Among the families containing important poisonous plants are the Anacardiaceae (poison ivy, sumach), Apocynaceae (dogbanes), Asclepiadaceae (milkweeds), Compositae (asters, cockleburs, sneezeweeds), Ericaceae (laurel), Leguminosae (lupines, loco weeds, milk vetches, vetches), Liliaceae (cannas, lilies), Loganiaceae (strychnin), Poaceae (grasses), Ranunculaceae (aconite, buttercups, larkspurs), Solanaceae (belladonna, henbane, nightshade, tobacco), Umbelliferae (hemlock), and Urticaceae (nettles). This takes no account of the many poisonous fungi, especially among the fleshy fungi, or mushrooms. The interested reader is referred to the comprehensive manual of poisonous plants by Pammel.

Fibers

The most important fibers are cotton, of the Malvaceae or mallow family, and flax, belonging to the Linaceae. In addition are hemp, of the Cannabinaceae; jute, representing the Tiliaceae; sisal, of the Amaryllidaceae, and abaca, or Manila hemp, belonging to the Musaceae or banana family.

Forest Materials

The two most important families producing forest materials in the temperate zone are Pinaceae, including pine, spruce, hemlock, fir, cypress, and juniper, and the Fagaceae, containing the oaks, beeches, and chestnuts.

Others of importance are Juglandaceae, walnuts and hickories; Aceraceae, maples; Fraxinaceae, ashes; Betulaceae, birches; and Poaceae, including the bamboos. In the tropics many other families furnish materials of high value.

Botany and Crop Improvement

We have seen that the improvement of our present crop plants, or the finding of new ones, is a most promising means of increasing the food supply. The wide taxonomic distribution of the plant families so important to human welfare is an earnest of the complexity of the botanic problem involved. All phases of botany, including taxonomy, morphology, physiology, ecology, genetics, and pathology, must contribute largely if substantial progress is to be made. Ecology and pathology are to have full discussion elsewhere on this program.

Taxonomy

The fundamental contribution of plant classification and description to human welfare has been the presentation of the vegetable kingdom as a fairly orderly series of evolving forms rather than a conglomeration of wonderful but unrelated organisms. The applications of this knowledge of relationships are many, varied, and valuable. Through such knowledge we are able to build up large plant industries with an assurance of success which otherwise would be impossible.

The classification of crop plants, when accomplished with the same precision and thoroughness which have been used in the case of wild species, will be of inestimable value to science and to humanity. The same principles will be applied, the same characters used, and the same results obtained. The *species* of crop plants already are fairly well described and classified. The present need is classification and description of the seemingly innumerable agronomic and horticultural *varieties* of these plants. The duty of taxonomic botany is to make it possible for plant workers everywhere to recognize crop varieties.

A classification of American wheat varieties now in manuscript has determined that the wheats passing under about 800 names actually represent about 200 distinct and recognizable varieties. While the proportion of synonyms in this instance may be no greater than the proportion in any other line of systematic botany, it must be remembered that the results in the case of wheat varieties are measured in bushels and not in bibliographies, in dollars and not in doubts. Such a classification of wheat varieties makes it possible to determine promptly that the so-called Superwheat of a Burbank is identical with the old and well-known Jones Winter Fife of New York and the Inland Empire, and that the only miracle about a "Miracle" wheat is the number of suckers it attracts.

Similar results are coming out of the application of botanic classification to varieties of oats and other cereal crops, and to cow peas, soy beans, cottons, sorghums, lettuce, beans, apples, plums, peaches, and every kind of crop plants. Some years ago the writer saw at one of the largest agricultural experiment stations in the United States a long series of plats of cereal varieties of which less than 50 percent were under the right varietal names. Of what value will be the published results, if the varietal names are wrongly applied?

Ten years ago, in his address as retiring president of the Botanical Society of Washington, Piper recorded his belief that fully 50 percent of the crop varieties published upon in varietal experiments were either untrue to name or unidentifiable. But how shall they become identifiable without adequate description and classification? And how shall they become adequately described and classified without botanists to study them?

Large numbers of important varieties of crop plants have been produced by the selection of pure lines, by the selection of mutations, and by the production and selection of hybrid forms. The intrinsic value of these important strains is great, but there is present the continual danger of their being lost by submersion among the many more or less similar varieties of the crop they represent. Careful botanic descriptions of the recognizable points of difference between them and the forms most closely related, accompanied by adequate illustration, should make it possible for crop growers to recognize these varieties with some degree of certainty.

Throughout the history of agriculture, unscrupulous dealers have substituted inferior material for superior when opportunity has occurred. Increased production of superior strains can be assured only when it is possible to detect substitutions, and this is possible only when all closely related forms are so well described as to be fairly identifiable by an intelligent layman.

Another opportunity of systematic botany in relation to crop plants lies in the finding and introduction of new material. Large portions of the earth still are not well known botanically. The plants of vastly larger areas have not been critically studied with reference to their usefulness in other lands. Some will be of direct and immediate service as food materials. Others will provide hardy or disease-resistant stocks for grafting purposes, while still a third series will possess characters valuable when transmitted through hybridization.

The Problem and the Challenge

To place varietal experimentation on a firm basis of accurately described and easily recognized material; to insure the identity of new and valuable strains; to prevent the faker from profiteering at the expense of crop producers; and to provide new plant materials from the four corners of the earth, is the greatly needed contribution of systematic botany to crop-plant production and so to human welfare.

The work previously done on the classification of varieties of crop plants and the introduction of new material has been the separate product of two different groups of workers, botanists and agronomists. You have heard the saying that no botanist will look at a cultivated plant, and no agronomist at a wild one. Granting the exaggeration, the saying is still too true.

A generation of botanists must be trained to appreciate the fundamental importance of full taxonomic knowledge of crop plants. They must recognize that characters sufficient to separate *species* of wild plants serve only to separate closely related field and garden *varieties* of domesticated plants. In dealing with the latter, they must be willing to forget Latin nomenclature, if need be, as a Latin terminology must be carried to the fifth, sixth, and seventh place in such crops as wheat or corn if one builds on the taxonomic foundations already laid. Likewise, a generation of agronomists must be produced which has had good foundation training in systematic botany, derived in large part from a study of crop plants.

I put the challenge squarely up to the botanical departments of our universities and our land-grant colleges alike to work together to produce such a generation of botanists. Equally squarely are the departments of agronomy in the land-grant colleges challenged to coöperate with the botanical departments in producing such agronomists. This challenge is to the institutions involved.

A similar challenge clearly lies before the present and future personnels engaged in the investigation of plants from both the agronomic and the botanic points of view. The obligation is upon them to coöperate, pooling their valuable resources of accumulated experience and information and expensive equipment in a common cause. Only by such coöperation can satisfactory progress be made in the attack on these problems so vital to the welfare of humanity.

Crop Physiology

At the present time, progress in crop improvement is waiting on a fuller knowledge of crop physiology. To the farmer, as to the agronomist, the value of crop varieties is measured in terms of their performance in pounds or bushels. We know by experimentation that one variety of any given crop yields better under a certain set of conditions than do other varieties of that crop, while under different conditions this same variety may be comparatively unproductive. We know that crops vary greatly in their comparative resistance to disease, to cold, to frost, to heat, to drought, to soil alkali, and to all the other unfavorable factors in the environment. In the same way, some varieties seem unable to stand prosperity. Given what apparently are very favorable conditions, they seem unable to make a proportionate response in production. These things we know, but what we do not yet know is why these things are so. That is the next and most immediate problem in crop improvement.

In the practice of medicine, the detailed study of the functioning of the various members of the human body has been held indispensable to a proper diagnosis of diseased conditions. In the case of even a single one of our most important crop plants, however, no such detailed study has been made. We attempt to acclimatize them in various parts of the world, to make them productive under a wide range of climatic conditions, and to breed them to produce forms with very specialized adaptations, without this fundamental knowledge of their relations, derived from adequate research.

The relative and actual importance of such external factors as light, air temperature, humidity, soil temperature, soil texture, and soil solution in their effect on the growing crop plant at different stages of growth, from germination to maturity, are very imperfectly known quantities today. Without doubt, the increasing determination of the values of these and other factors will have a profound influence on the practices of crop production and ultimately on the quantity and quality of the product.

The recent discovery by Garner and Allard, of the effect on plant growth caused by varying the duration of the daily light period, not only is shaking the foundation of our theories and opens leads toward many unsolved problems, but is highly suggestive of results that will be obtained when other and equally fundamental researches are made in the realm of crop physiology. Some of the lines along which such research should be directed have been mentioned. Next to light, the fundamental factors are temperature, water, and food.

Several unrelated studies of temperature relations have been made, but research to date has touched only the fringe of this problem. Temperature studies are vital to such problems as crop adaptations, including the extension of the areas of fall-sown crops, as against spring-sown; the comparative development of root and shoot and the speed of development of the resulting plant, and the determining of the conditions under which plants may escape or resist the attacks of soil-infecting and other fungi.

The study of water relations is of very high importance. Studies in the water requirements of several important crop plants, as revealed through transpiration measurements, have been conducted in recent years by Briggs and Shantz and by Montgomery and Kiesselbach, but these are only a good beginning compared with the research that is needed.

Investigation of the duty of water in irrigation is much more a problem for the plant physiologist than for the irrigation engineer. The first phase is the determination of the effect of applying water, at different times and in varying quantities, on the comparative and actual development of the roots, vegetation, and fruits of crop plants. The second phase is the possibility of increasing total crop production by making the present supply of irrigation water cover much more than the present number of acres. It is conceivable that reducing the quantity of water by one half might reduce acre production by only two or three tenths and permit irrigation of twice the present acreage. Two acres of irrigated wheat yielding 35 bushels each may be more valuable to humanity and just as profitable to the grower as one acre yielding 50 bushels. It is not unthinkable that one day we shall see governments exercising the right of eminent domain to accomplish such results through reducing existing water rights.

Studies in plant nutrition long have been known to be of fundamental importance. The chief difficulty in such research has been to control experimental conditions and at the same time to approximate natural conditions. Solution cultures permit controlled conditions but give only suggestive results. Fertilizer plats approximate natural environment, but are conducive to confusing interpretations. The gulf between the two may be bridged by continued refinement of method and interpretation.

Studies in the physiology of the development of seeds and fruits in our major food plants, such as the cereals, are of the utmost concern. The period of vegetative growth may be prolonged over several months, but usually the formation and maturing of seeds takes place in the brief period of two to four weeks. Obviously, this is an important and perhaps even critical period in the life of the plant, from the economic standpoint. Physiology can help to show what tillage, or irrigation, or fertilizer practices during or just previous to this period, will influence directly the quantity and quality of the product.

Some preliminary studies in the deposit of protein and starch in developing wheat kernels were made several years ago in the state of Washington by Dr. Thatcher and his associates. Dr. Harlan, of the Office of Cereal Investigations, U. S. Department of Agriculture, is now publishing a series of papers dealing with some phases of the development of the barley kernel. Such studies are but the forerunners of what is required as a foundation for a better knowledge of the behavior of our crop plants at this critical period in their development.

In a present study of soil-infesting rots of the corn plant, coöperative between the Office of Cereal Investigations and the Indiana Agricultural Experiment Station, Funk Brothers Seed Company, and other agencies, some striking physiologic factors have been found to be involved in what was supposed to be purely a pathologic problem. Changes occurring within the plant result in a deposit of harmful metals and consequent severe injury to the plant. Research on the cause of the abnormal metabolism emphasizes how little we know of the functioning of the corn plant in health. And yet here is a crop worth several billions of dollars annually in our own country alone!

During the last half century there has been no lack of attention to the subject of plant physiology. I draw here, however, a clear distinction between plant physiology and crop physiology, because plant physiology has been restricted very largely to studies of wild species. Physiological research in our great state and privately-endowed American universities has not lacked equipment and encouragement. Splendid results have been obtained in such research, but until recently a scanning of the titles of theses submitted in connection with the granting of doctorate degrees warrants the statement that rarely has a candidate undertaken research on a domesticated plant.

The importance of fuller knowledge of crop physiology, in relation to our national welfare, warrants these universities more and more in devoting their magnificent resources of men and equipment to such research. There is no reason why this should not be done in coöperation with plant workers in state experiment stations or in the research bureaus of the U. S. Department of Agriculture. I am sure that the universities would be met more than half way if such coöperation were proposed. There is a large enough field for all, and human need does not warrant the self-imposed exclusion of any agency capable of giving effective assistance in the solution of the problems involved.

Genetics

Some twenty years ago, the rediscovery and interpretation of the remarkable work of Gregor Mendel created a new branch of plant physiology and ushered in a new epoch in plant improvement. As a result, important results are being achieved in two opposite directions. Looking backward, new light is being thrown on the origin of existing plant forms. Looking forward, our knowledge of somatic behavior is being used in the creation of new forms of high intrinsic or potential value.

Genetic studies hold the greatest possibilities for improvement in crop production. The knowledge of the plant sources from which have been developed such tremendously variable and important crop plants as corn or wheat would greatly aid in our understanding of how to proceed in obtaining forms with needed characters. Just as fast as physiologic research can show the nature of such desirable characters as resistance to rust, smut, cold, drought, and the many other pests and unfavorable influences which reduce crop production, genetics will help in combining existing varieties to produce other better adapted ones with the desired characters. At the same time undesirable characters may be eliminated.

Prophets of the New Order

I have been interested to discover what the leaders of botanical thought were emphasizing a quarter-century ago. On consulting the addresses presented about that time by the retiring presidents of the Botanical Society of America and the retiring chairmen of Section G of the American Association, I was particularly interested to find that already they were foreshadowing or openly proclaiming the importance of the economic phases of botany. It was especially interesting to note that three such veterans as Doctors Coulter, Trelease, and Galloway, as well as others, should have had this viewpoint in common. I cite these three especially because the first has devoted his entire career to so-called pure botany, the second has divided his affiliation between the wild and the cultivated plants, while the third has been engaged continuously on various phases of applied botany.

Turning then to very recent pronouncements, I was especially gratified to note the point of view of Dr. Coulter in his address as retiring president of the American Association in December, 1919. In this address, entitled "The Evolution of Botanical Research," he noted three botanical tendencies, as follows:

1. To attack problems fundamental to some important practice,
2. To realize that botanic problems are synthetic, and
3. To recognize that plant structures are not static.

He noted also three important features of future botany, namely:

1. Broader training to be required of botanical workers,
2. More extensive coöperation in research, and
3. Better development of experimental control.

The addresses of Dr. Flexner, three days ago, on "Twenty-five Years of Bacteriology," and of Dr. Pammel, today, on "Some Economic Phases of Botany," are striking records of achievement in applied botany but were given in your hearing and need no discussion here.

Aesthetic Welfare

So far all our discussion has been of the relation of crop-plant botany to material welfare. Its relation to the aesthetic and spiritual welfare of man is less obvious, though perhaps not so much less potent as some may think. At any rate, it is impossible to develop this phase adequately in the limits of the present paper.

When the immortal author of Thanatopsis advised those sick in mind and spirit to "go forth under the open sky and list to nature's teachings," we are sure that the still small voice of useful plants was not excluded from the curative agencies. That scientific worker is indeed defrauded who does not get both mental exhilaration and spiritual uplift from contemplation, in crop plants, of the riotous beauty of floral color, the seductive fragrance of myriad blooms, the marvelous intricacies of structure, and the wonders of adaptation, or from the quietness of far-stretched fields of grain or cotton, and the majesty of towering forest forms, saying, in the latter case, with the dead soldier, Joyce Kilmer, "But only God can make a tree."

In Conclusion

The fundamental botanic requirements in crop production are to know what we now have, to find what exists elsewhere, and to use both in creating something better than either.

To know what we have requires botanic description, classification, and illustration, and a study of plant functioning. To find what exists elsewhere and to predict where it may be useful requires expert knowledge of plant relationships and plant ecology. To create the best requires intimate genetic knowledge, and a visualizing of the plant that is to be in terms of the characters of plants that are. To no more worthy tasks can botanists devote their best endeavors.

Office of Cereal Investigations,
U. S. Department of Agriculture

CORRELATIONS BETWEEN ANATOMICAL CHARACTERS IN THE SEEDLING OF PHASEOLUS VULGARIS

J. Arthur Harris, Edmund W. Sinnott, John Y. Pennypacker, and G. B. Durham

(Received for publication January 17, 1921)

Introduction

In an earlier paper[1] we traced the course of the vascular bundles throughout the dimerous and trimerous seedlings of *Phaseolus vulgaris* and measured the variation occurring at different levels.

The chief results of that paper were (*a*) the demonstration of the profound differentiation of dimerous and trimerous seedlings in their internal (vascular) as well as in their external characters, (*b*) the demonstration that the number of bundles at a given level in the seedling is a highly variable rather than a constant character, and (*c*) that the various organs or regions of the plant body (particularly, in the present case, those which are separated by the vascular anastomoses at the cotyledonary node) differ widely in the magnitude of their variability as to bundle number.

In this paper we propose to consider in quantitative terms the degree of interrelationship between the vascular structures in the different regions of normal and abnormal seedlings. The results of such an investigation will evidently be of considerable morphological interest, since many of the problems of organic form are fundamentally problems of correlation.

Two morphological problems at once present themselves for consideration:

First, is there a high correlation between the vascular topography of two different levels of the same internode, *i.e.*, is the number of vascular bundles constant throughout the length of an internode or is there more or less extensive splitting or anastomosis within the length of such a conventional morphological unit?

Second, is there a definite correlation between the vascular topography below a node and the vascular topography above it, or is the vascular system so fully reorganized at the nodal anastomosis of bundles that, in bundle number, successive internodes are practically independent of one another?

With the present material, these questions may be answered by determining the coefficients of correlation for bundle number between (1) the base and the mid-region of the hypocotyl, and (2) between the various levels of the hypocotyl and the mid-region of the epicotyl. It is these problems which we propose first to consider. We shall also compare the normal and abnormal seedlings as to the correlations which they exhibit, and shall touch briefly on the problem of the correlation between bundle number in seedlings from the same parent plant.

---

[1] Harris, J. Arthur, Sinnott, E. W., Pennypacker, John Y., and Durham, G. B. The vascular anatomy of dimerous and trimerous seedlings of *Phaseolus vulgaris*. Amer. Jour. Bot. **8**: 63–102. 1921.

The frequency distributions of bundle number are in many cases of very narrow range and very skew. There has, therefore, been considerable question as to the formulae to be employed. It has seemed best, for various reasons which need not be detailed here, to employ the usual method of product-moment correlation.

Presentation and Analysis of Data

The series of data considered here are in large part the same as those discussed in our earlier paper, but have in some cases been supplemented by the examination of additional sections. These have been included when the dimerous and trimerous seedlings were not true siblings. In lines 75, 93, and 98, the series compared were obtained from the same mothers. In so far as the data are the same as those used earlier, the variation constants for the different characters have already been presented and discussed and require no further comment here. The data from which measures of interrelationship may be computed are given in our fundamental tables A to L. We have, therefore, merely to deduce and discuss the correlation coefficients.

Correlation between Bundle Number at Different Levels in the Same Internode

We first turn to the problem of the relationship between the number of bundles—primary double bundles, intercalary bundles, and total bundles—at the base of the hypocotyl and the number in the central region of the hypocotyl. The reader who cares to do so may reconstruct the 24 correlation tables necessary for a consideration of these relationships from our fundamental tables A–L.

Table i. *Coefficients of correlation between number of primary double bundles, number of intercalary bundles, and total bundles at base of hypocotyl, and number of bundles in central region of hypocotyl*

Character of Seedlings and Line	N	Correlation for Primary Double Bundles r_{ph}		Correlation for Intercalary Bundles r_{ih}		Correlation for Total Bundles r_{bh}	
Trimerous							
Line 75	142	+.378±.049	7.79	+.329±.051	6.51	+.649±.033	19.8
Line 93	155	+.233±.051	4.55	+.204±.052	3.92	+.469±.042	11.1
Line 98	183	+.321±.045	7.17	+.253±.047	5.42	+.586±.033	17.9
Line 139	106	+.417±.054	7.71	+.097±.065	1.50	+.531±.047	11.3
Line 143	221	+.556±.031	17.8	+.305±.041	7.40	+.753±.020	38.3
Dimerous							
Line 75	142	+.362±.049	7.35	+.668±.031	21.3	+.797±.021	38.0
Line 93	155	+.641±.032	21.0	+.390±.046	8.50	+.753±.023	32.2
Line 98	183	+.666±.028	24.0	+.555±.035	16.1	+.786±.019	41.4
Line 139	305	+.344±.034	10.1	+.898±.008	119.7	+.925±.006	168.3
Line 143	420	+.530±.023	22.5	+.634±.020	32.2	+.802±.011	68.5

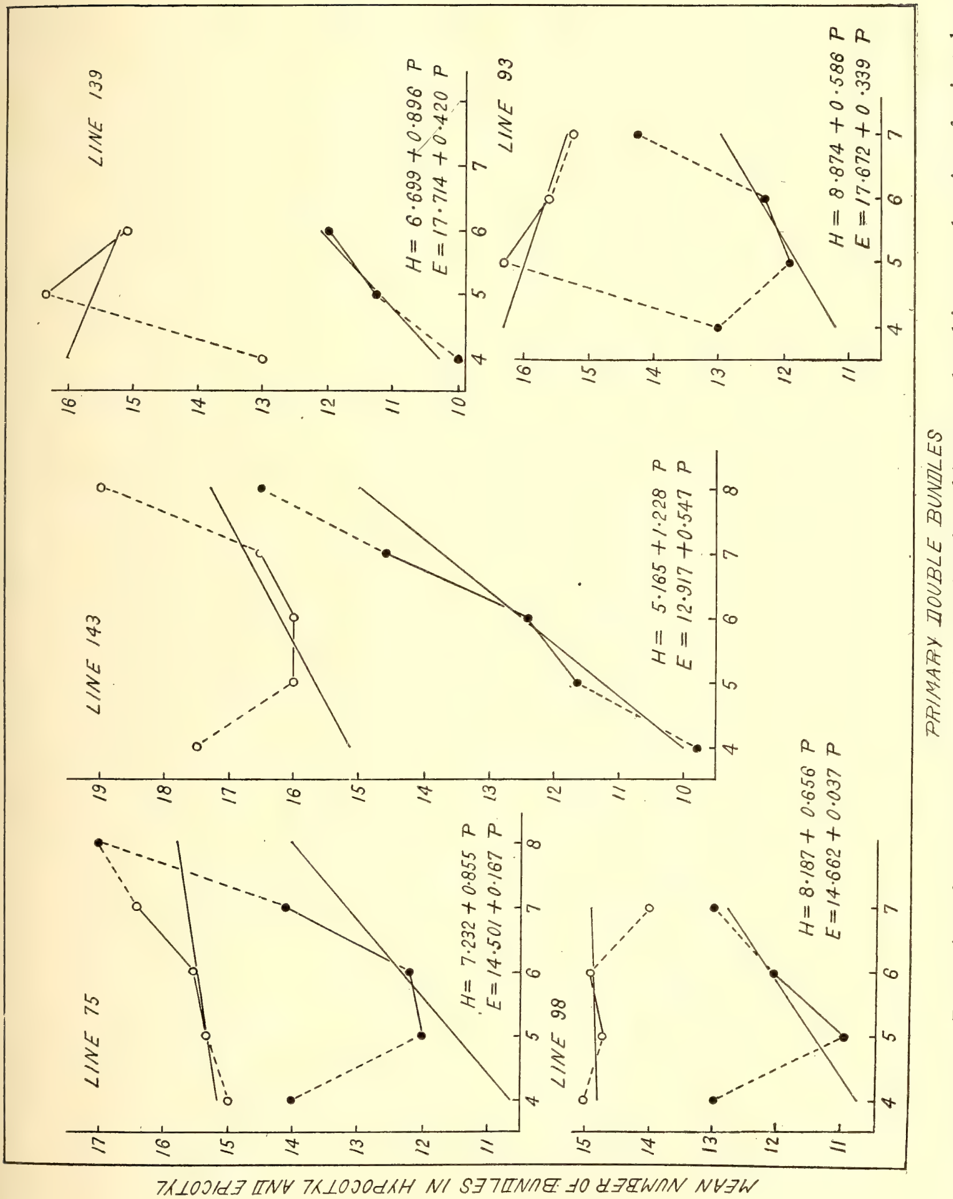


DIAGRAM I. Regression of number of bundles in central region of hypocotyl and in central region of epicotyl on number of primary double bundles at base of hypocotyl in trimerous seedlings. Empirical means represented by solid dots for hypocotyl and by circles for epicotyl.

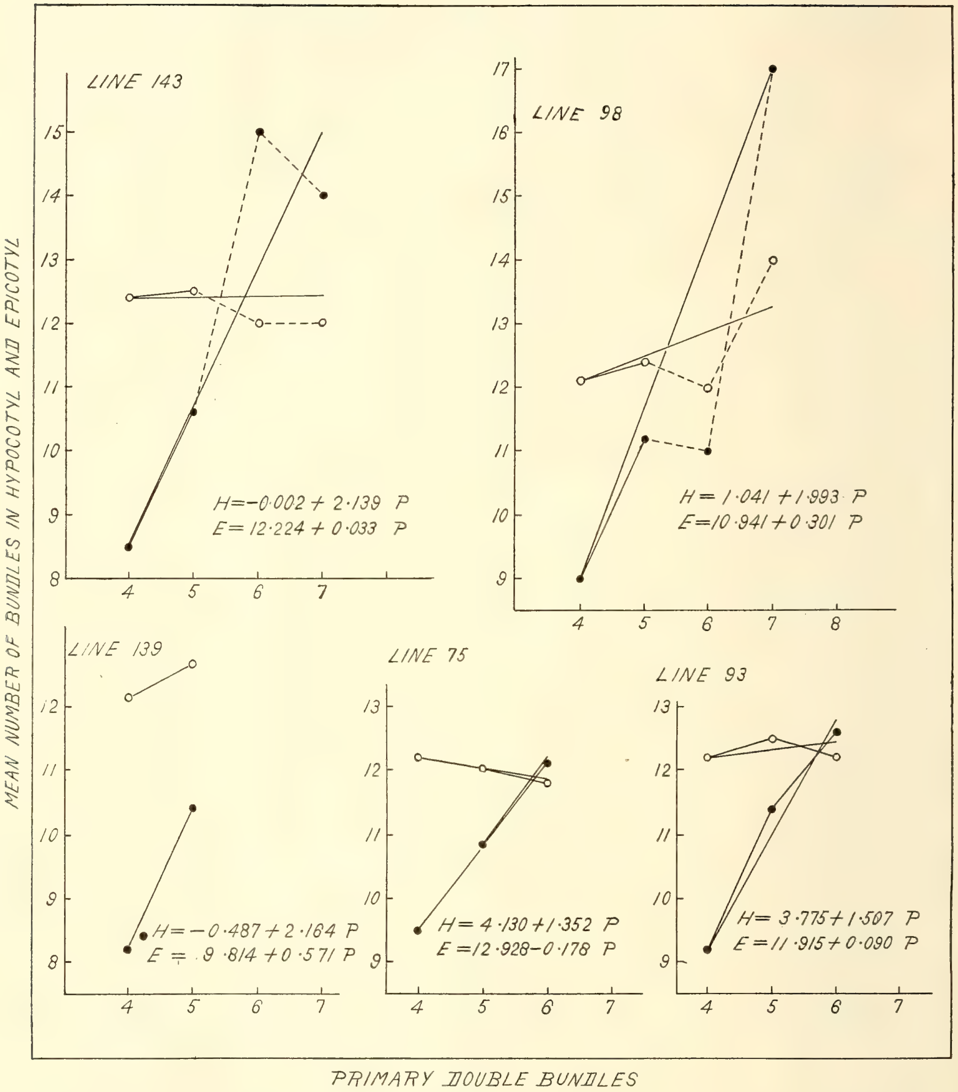


Diagram 2. Regression of number of bundles in central region of hypocotyl and in central region of epicotyl on number of primary double bundles at base of hypocotyl in dimerous seedlings. Empirical means represented by solid dots for hypocotyl and by circles for epicotyl.

The correlation coefficients between the two classes of bundles which have been recognized at the base of the hypocotyl and the total number of basal bundles (*i.e.*, the sum of the number of primary double bundles and the number of intercalary bundles in the base of the hypocotyl) and the number in the central region of the hypocotyl, appear in table 1.

The correlations are without exception positive in sign and of a material order of magnitude. They have been expressed in terms of regression on diagram 1 for trimerous seedlings and on diagram 2 for dimerous seedlings of the five lines.[2]

Primary Double Bundles and Mid-region of Hypocotyl

The constants showing the relationship between number of primary double bundles and number of bundles in the central region of the hypocotyl, r_{ph}, are shown in the first section of table 1. They are positive and statistically significant in all cases in both dimerous and trimerous seedlings. The average value of the coefficient for the five lines investigated is +.3810 for trimerous seedlings and +.5086 for dimerous seedlings.

Diagram 2 shows that in the case of the normal plants of lines 75, 93, and 143 a straight line represents very well indeed the changes in the mean number of bundles in the hypocotyl with variations in the number of primary double bundles at the base of the hypocotyl. In line 98 the agreement is apparently not so good. This is, however, attributable to the fact that of the 183 plants only two have more than 5 primary bundles. Of these two, one plant is recorded as having 8, which is twice the normal number. In line 139 only plants with two classes of seedlings, those with 4 or 5 primary bundles, are available, and since the regression line must connect the two means it is idle to discuss linearity of regression.

Turning to the trimerous plants represented in diagram 1, we note that because of the small number of plants with other than 5 or 6 primary double bundles the distribution of the empirical means is very irregular indeed. There is some suggestion of non-linearity, but the number of seedlings in the more extreme classes is so small for every line that little stress is to be laid upon them.

In both normal and abnormal plants the slope of the regression line is rather steep, showing a material change in the number of bundles in the central region of the hypocotyl with variations in the number of primary double bundles at the base of the hypocotyl.

Intercalary Bundles and Mid-region of Hypocotyl

The correlation between the number of intercalary bundles and the total number of bundles in the hypocotyl, r_{ih}, are shown in the second

---

[2] The equations on the diagrams show the regression of the number of bundles in the central region of the hypocotyl, H, and in the central region of the epicotyl, E, on the number of primary double bundles, P, at the base of the hypocotyl. The empirical means for the hypocotyl are represented by solid dots, while those of the epicotyl are represented by circles. In both cases the empirical mean number of bundles for the same organ are connected by solid lines when the number of sections averaged was five or more, but by broken lines when the number available was four or less. Fortunately for purposes of graphical representation, the mean number of bundles in both hypocotyl and epicotyl can be drawn on the same diagram. Only the lower lines in each of the five panels of the two diagrams require consideration for the moment.

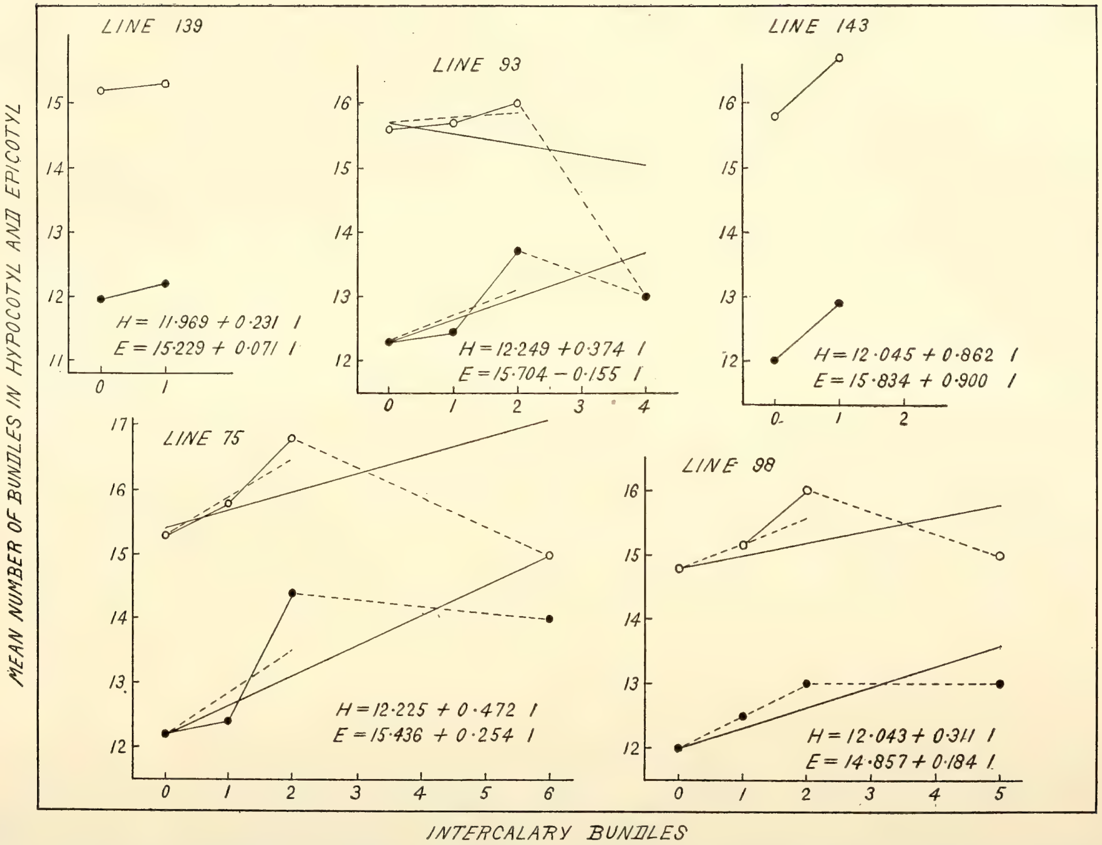


DIAGRAM 3. Regression of number of bundles in central region of hypocotyl and in central region of epicotyl on number of intercalary bundles at base of hypocotyl in trimerous seedlings. Empirical means represented by solid dots for hypocotyl and by circles for epicotyl.

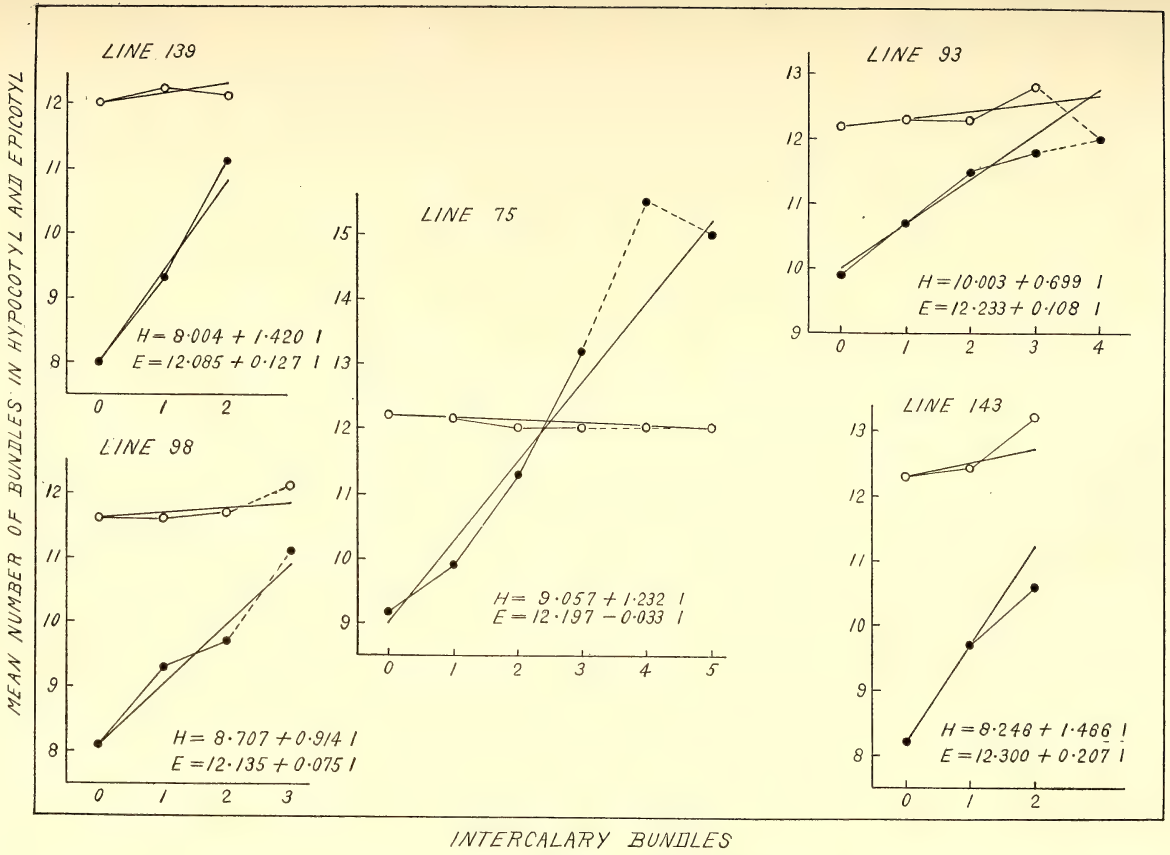


Diagram 4. Regression of number of bundles in central region of hypocotyl and in central region of epicotyl on number of intercalary bundles at base of hypocotyl in dimerous plants. Empirical means represented by solid dots for hypocotyl and by circles for epicotyl.

section of table 1. The straight-line equations showing the regression of the number of bundles in the central region of the hypocotyl are recorded and represented graphically on diagram 3 for trimerous seedlings and on diagram 4 for dimerous seedlings. These diagrams, like the two preceding, also give the regression equations and their graphic representation for the epicotyl which will be discussed in a subsequent section.

The correlation coefficients are positive in all cases, and with one exception may be considered statistically significant. They show, however, a considerable irregularity from line to line, presumably because of the varying range and distribution of number of intercalary bundles. The average value of the coefficient is +.2376 for trimerous seedlings and +.6290 for dimerous seedlings.

Turning to the graphs, we may note that for the dimerous plants the agreements between the empirical and the theoretical means are very good indeed. The slope of the lines for the hypocotyl is very steep.

The graphs for the trimerous plants show far greater irregularities because of the generally small number of the strands but the occasional occurrence of plants with a larger number. Reference to the tables will show that in line 75 there is one seedling with 6 intercalary bundles whereas the remaining 141 seedlings have only 0, 1, or 2 intercalary bundles. In line 93 there is only one seedling with more than 2 intercalary bundles and it has 4. In line 98 all the frequencies with two exceptions fall on 0 or 1 intercalary bundle.

The correlations and equations have been recalculated, leaving these extreme cases out of account. The regression straight lines based on all the material are represented by solid lines. Those in which the extreme class were omitted are represented by broken lines.[3] The removal of these aberrant cases has increased the agreement between the observed and the theoretical means but the fit is still far from satisfactory. The only conclusion which can be drawn from these diagrams is that there is a considerable degree of positive correlation between the number of the intercalary bundles and the number of bundles in the hypocotyl.

Total Basal Bundles and Mid-region of Hypocotyl

The correlations between total bundles (primary double bundles + intercalary bundles) at the base of the hypocotyl and the number of bundles in the central region of the hypocotyl, r_{bh}, are shown in the third section of table 1. The straight-line regression equations are given and represented graphically as the lower figures in each panel of diagram 5 for trimerous seedlings and diagram 6 for dimerous seedlings.

As might be expected on *a priori* grounds, these coefficients agree with those for primary double bundles and for intercalary bundles in sign, and

---

[3] For the curtailed series the regression equations are: Line 75, $H = 12.194 + 0.654 I$; Line 93, $H = 12.238 + 0.462 I$; Line 98, $H = 12.030 + 0.473 I$.

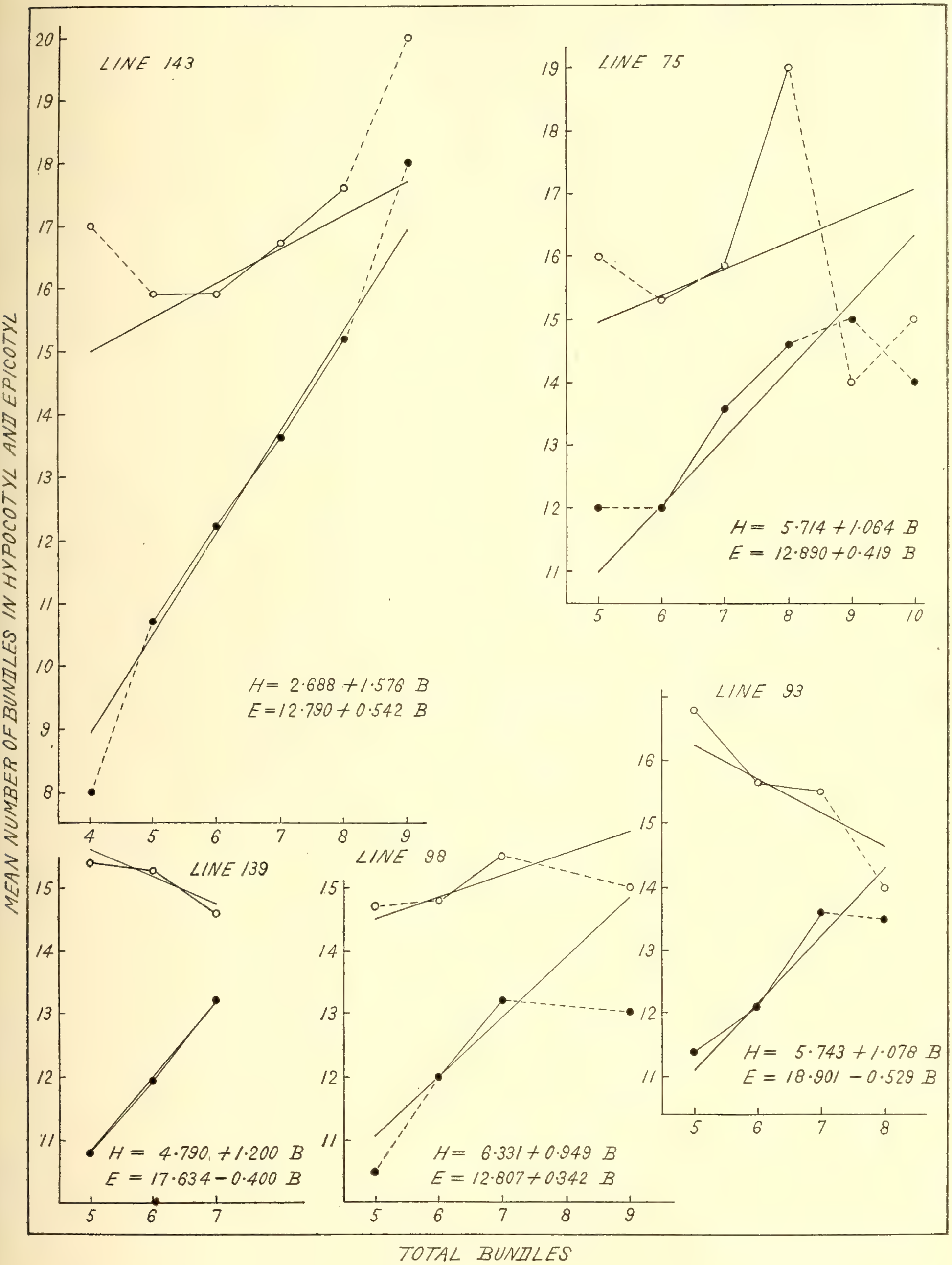


Diagram 5. Regression of number of bundles in central region of hypocotyl and in central region of epicotyl on total number of bundles at base of hypocotyl in trimerous seedlings. Empirical means represented by solid dots for hypocotyl and by circles for epicotyl.

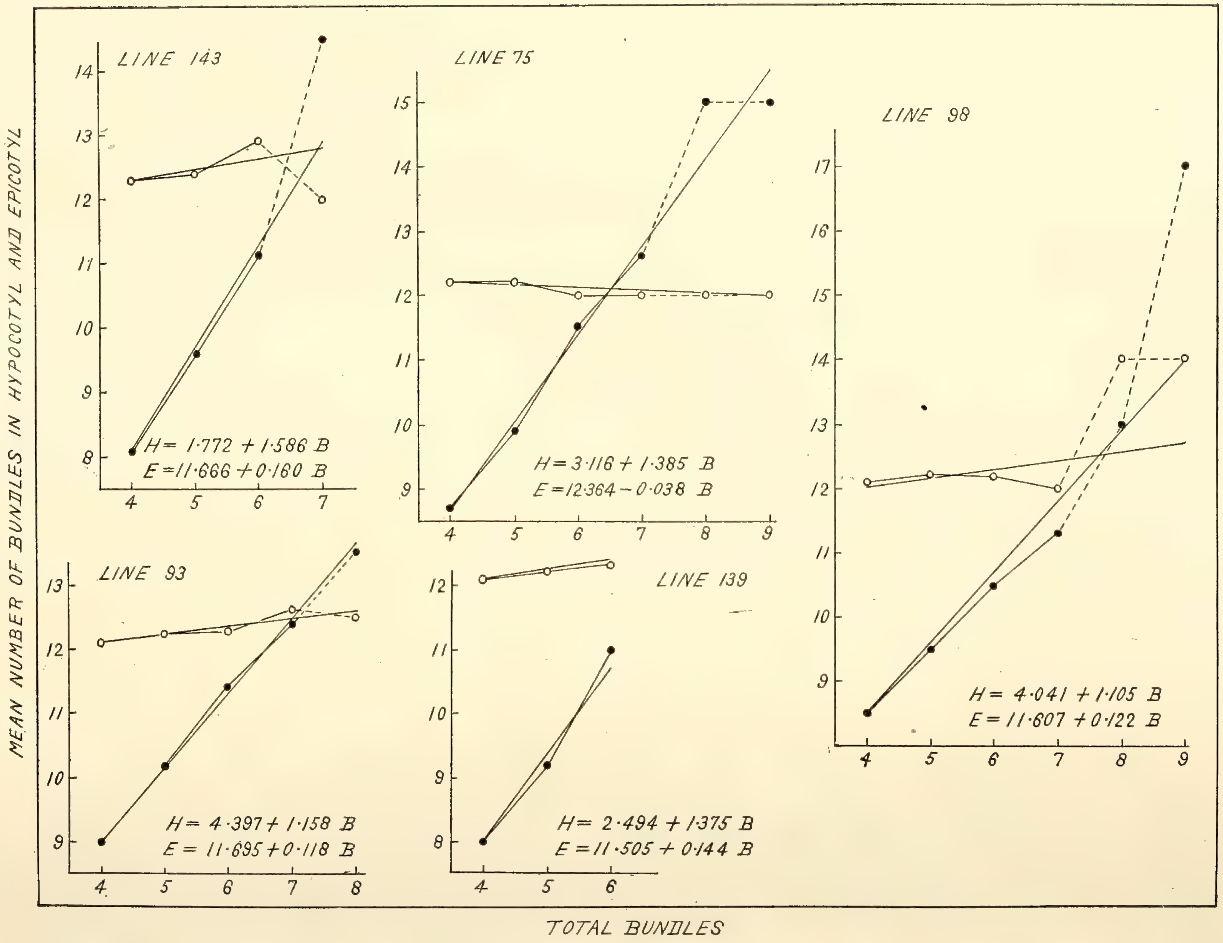


Diagram 6. Regression of number of bundles in central region of hypocotyl and in central region of epicotyl on total number of bundles at base of hypocotyl in dimerous seedlings. Empirical means represented by solid dots for hypocotyl and by circles for epicotyl.

are in general somewhat higher than those for either of these two classes. The average value of the 5 coefficients for trimerous seedlings is +.5976 while that for dimerous seedlings is +.8126.

Turning to the diagrams, we note that the straight lines and the empirical means are in excellent agreement, considering the small number of seedlings, in the case of the normal plants, but show greater irregularities in the case of the abnormal plants. This is due to a considerable extent to the greater concentration of the frequencies into two classes in the case of the trimerous seedlings.

We may now consider the relative magnitudes of the three correlations which we have been studying. Table 2 shows the differences existing between the various correlations, *i.e.*, the possible differences between the correlation for primary bundles and hypocotyledonary bundles, r_{ph}, for intercalary bundles and hypocotyledonary bundles, r_{ih}, and for total bundles at the base of the hypocotyl and hypocotyledonary bundles, r_{bh}.

TABLE 2. *Comparison of correlations between the various types of bundles at the base of hypocotyl and the number of bundles in the central region of hypocotyl*

Character of Seedlings and Line	$r_{bh} - r_{ph}$		$r_{bh} - r_{ih}$		$r_{ph} - r_{ih}$	
Trimerous						
Line 75	+.271±.059	4.59	+.320±.061	5.25	+.049±.071	0.69
Line 93	+.236±.066	3.58	+.265±.067	3.95	+.029±.073	0.40
Line 98	+.265±.056	4.73	+.333±.057	5.84	+.068±.065	1.05
Line 139	+.114±.071	1.61	+.434±.080	5.43	+.320±.084	3.81
Line 143	+.197±.037	5.32	+.448±.046	9.74	+.251±.051	4.92
Dimerous						
Line 75	+.435±.053	8.20	+.129±.037	3.49	−.306±.058	5.28
Line 93	+.112±.040	2.80	+.363±.051	7.11	+.251±.056	4.48
Line 98	+.120±.033	3.64	+.231±.040	5.78	+.111±.045	2.47
Line 139	+.581±.035	16.6	+.027±.010	2.70	−.554±.035	15.8
Line 143	+.272±.026	10.5	+.168±.022	7.64	−.104±.030	3.47

For both dimerous and trimerous seedlings, the correlations between the total bundles at the base of the hypocotyl and the number of bundles in the central region of the hypocotyl are higher throughout than those for either of the two separate types of bundles (primary bundles and intercalary bundles) individually considered. In general, the differences are sufficiently large in comparison with their probable errors to be considered statistically significant.

The comparison of the magnitudes of the correlations between numbers of primary double bundles and of vascular elements at higher levels, and between numbers of intercalary bundles and of vascular elements at higher levels, shows that in 7 of the 10 comparisons the closer correlation of hypocotyledonary bundles is with the primary double bundles.

Lines 75, 139, and 143 present exceptions. In the normal plants of these lines the correlation between intercalary bundles and total bundles in the hypocotyl is apparently significantly higher than that between primary double bundles and total bundles in the hypocotyl.[4]

The fact that the number of bundles in the central region of the hypocotyl is about equally correlated with the number of primary double bundles and with the number of intercalary bundles at the base of the hypocotyl shows that both types of bundles are of about equal significance in determining the number of bundles in the central region of the hypocotyl.

From the foregoing discussion it is clear that there is a rather close relationship between number of bundles at the base and the number in the central region of the hypocotyl. This might, we believe, have been expected on *a priori* morphological grounds. The interesting feature of the results seems to be that the correlations are not larger. The results show that there is a very large amount of irregularity in the division of primary strands or in the formation of intercalary bundles, or in both, as one passes the short distance from the base of the hypocotyl to the central region.

Correlation between Bundle Number in Different Internodes

The data available for a consideration of the problem of the correlation between bundle number in adjacent internodes cover (A) the correlation between the three classes of bundles at the base of the hypocotyl [primary double bundles (p), intercalary bundles (i), and total bundles (b)] and the number of bundles in the central region of the epicotyl; and (B) the correlation between the number of bundles in the central region of the hypocotyl and in the central region of the epicotyl.

(A) The coefficients showing the relationship between the numbers of primary double bundles, of intercalary bundles, and of total bundles at the base of the hypocotyl, and the number of bundles in the central region of the epicotyl, appear in table 3.

The regression equations showing the actual change in number of epicotyledonary bundles associated with variation in the number of primary double bundles are given and are represented with the empirical means of arrays on diagram 1 for trimerous plants and on diagram 2 for dimerous plants.

The graphs for the theoretical lines and the empirical means for the number of bundles in the epicotyl of both normal and abnormal plants show relatively little relationship between the number of bundles at the base of the hypocotyl and the number in the epicotyl. The differences in the slope of the lines for primary basal bundles and the number of bundles in central regions of hypocotyl and epicotyl show in a most striking manner the differences between correlations for groups of bundles lying on the same side and those lying on different sides of the nodal complex.

---

[4] In line 75 the range of primary double bundles is only 3 while that of intercalary bundles is 6. In line 139 the primary double bundles fall in two classes only, with but 3 of the 305 frequencies on 5 as compared with 302 on 4 bundles. The correlation coefficient in such a case can have but little value. In line 143 practically all of the primary double bundles fall in two classes while the intercalary bundles are limited to three classes.

Irregularity of results must be expected under such conditions.

(1) The correlation coefficients between primary double bundles and number of bundles in the epicotyl, r_{pe}, as set forth in the first section of table 3, are in part positive and in part negative in sign. For the most part they can not be considered statistically significant. The average value of those for trimerous seedlings is −.0226 while that for dimerous seedlings is +.0768.

(2) For the correlation between the number of intercalary bundles and the number of bundles in the epicotyl, r_{ie}, shown in the second section of table 3, the coefficients are not in general certainly significant in comparison with their probable errors. Two of the ten are indeed negative in sign. The coefficients for line 143 in both trimerous and dimerous seedlings and possibly that for line 139 in the dimerous seedlings may be significant. The fact that eight of the ten coefficients are positive suggests that there is a slight relationship between the number of intercalary bundles at the base of the hypocotyl and the number of vascular elements in the central region of the epicotyl. The general average is +.0780 for the trimerous and +.0954 for the dimerous.

TABLE 3. *Coefficients of correlation between number of primary double bundles, number of intercalary bundles and total number of bundles at base of hypocotyl, and number of bundles in central region of epicotyl*

Character of Seedlings and Line	N	Correlation for Primary Double Bundles r_{pe}		Correlation for Intercalary Bundles r_{ie}		Correlation for Total Bundles r_{be}	
Trimerous							
Line 75	142	+.053±.056	0.93	+.126±.056	2.27	+.182±.055	3.33
Line 93	155	−.087±.054	1.61	−.055±.054	1.01	−.148±.053	2.79
Line 98	183	+.008±.050	0.70	+.070±.050	1.42	+.099±.049	2.01
Line 139	106	−.105±.064	1.63	+.016±.065	0.25	−.095±.065	1.47
Line 143	221	+.018±.045	0.40	+.233±.043	5.44	+.190±.044	4.34
Dimerous							
Line 75	142	−.115±.050	2.07	−.043±.057	0.75	−.054±.056	0.96
Line 93	155	+.084±.054	1.55	+.132±.053	2.48	+.167±.053	3.16
Line 98	183	+.239±.047	5.08	+.109±.049	2.21	+.205±.048	4.29
Line 139	305	+.164±.038	4.37	+.145±.038	3.84	+.175±.037	4.68
Line 143	420	+.012±.033	0.38	+.134±.032	4.15	+.121±.032	3.73

This suggestion is only slightly strengthened by inspection of the two sets of diagrams on which the regression equations are presented and drawn with the empirical means. Diagram 3 pictures the results for trimerous seedlings while the comparable representations for dimerous seedlings are shown on diagram 4. These show that while the slope showing the change in the number of bundles in the hypocotyl associated with variations in the number of intercalary bundles at the base of the epicotyl is very steep, it is practically nothing for the epicotyl, thus indicating a very close relationship in the former case but the practical absence of interdependence in the latter.

As explained above (p. 346), the slopes for the trimerous seedlings are very greatly influenced by certain aberrant individuals. When these are removed we obtain the equations represented by the broken lines in the figures.[5] The results for the relationship between the number of intercalary bundles and the number of bundles in the epicotyl indicate a positive correlation in all 3 cases when the one extreme plant is removed.

(3) The coefficients of correlation between total bundles (double bundles plus intercalary bundles) at the base of the hypocotyl and the number of bundles in the central region of the epicotyl, r_{be}, are shown in the third section of table 3, and are represented graphically in terms of regression in the upper figures of each panel of diagram 5 for trimerous seedlings and of diagram 6 for normal seedlings. The very gentle slope and the differences in direction of the lines for the epicotyl of the trimerous plants, together with the irregularity of the empirical means, serve to emphasize the slightness of the relationship between total bundles at the base of the hypocotyl and the number of bundles in the central region of the epicotyl. In the normal plantlets the means are less irregularly distributed about the theoretical lines, but the slope of the lines is very slight, and in one case the regression slope has the negative sign.

Turning to the correlation constants for more direct numerical comparison, we note that three of the ten constants are negative. The general average is $+.0456$ for the trimerous and $+.1228$ for the dimerous seedlings.

Looking back over diagrams 1–6, one cannot but be impressed by the difference in the slope of the lines showing the changes in number of bundles in the hypocotyl and in the epicotyl respectively associated with variations in the number of bundles at the base of the hypocotyl. The lines for the hypocotyl, without exception, indicate an increase in the number of bundles in the central region of the hypocotyl with an increase in the number of bundles at the base of the hypocotyl. The lines for the epicotyl occasionally show a decrease. Furthermore, the slopes of the lines for the hypocotyl are in general conspicuously steeper—thus indicating closer dependence upon the number of basal bundles—than those for the epicotyl.

Turning to table 4 for a numerical comparison of the correlations between the systems of bundles on the same side and on different sides of the cotyledonary node, we note that without exception the coefficients of correlation measuring the interrelationship between the number of vascular elements at the base of the hypocotyl and in the central region of the epicotyl are markedly lower than those measuring the correlation between the number of vascular elements in the base of the hypocotyl and in the central region of the hypocotyl.

(B) We now have to consider the problem of the correlation between the numbers of bundles in the central regions of the hypocotyl and of the

---

[5] When the extreme cases are omitted the equations are: Line 75, $E = 15.378 + 0.591 I$; Line 93, $E = 15.670 + 0.096 I$; Line 98, $E = 14.840 + 0.394 I$.

TABLE 4. *Differences between correlations for three classes of bundles at base of hypocotyl and the number of bundles in the central regions of hypocotyl and epicotyl, respectively*

Character of Seedlings and Line	$r_{pe} - r_{ph}$		$r_{ie} - r_{ih}$		$r_{be} - r_{bh}$	
Trimerous						
Line 75........	−.325±.074	4.39	−.203±.075	2.71	−.467±.064	7.29
Line 93........	−.320±.074	4.32	−.259±.075	3.45	−.617±.068	9.07
Line 98........	−.313±.067	4.67	−.183±.069	2.65	−.487±.059	8.25
Line 139.......	−.522±.084	6.21	−.081±.092	0.88	−.626±.080	7.83
Line 143.......	−.538±.055	9.78	−.072±.059	1.22	−.563±.048	11.7
Dimerous						
Line 75........	−.477±.070	6.81	−.711±.065	10.9	−.841±.059	14.3
Line 93........	−.557±.062	8.98	−.258±.070	3.69	−.586±.057	10.3
Line 98........	−.427±.055	7.76	−.446±.060	7.43	−.581±.052	11.2
Line 139.......	−.180±.051	3.53	−.753±.039	19.3	−.750±.037	20.3
Line 143.......	−.518±.040	12.9	−.500±.037	13.5	−.681±.033	20.6

epicotyl of the plant. The correlation surfaces are given in tables A–L. The results are set forth in table 5.

TABLE 5. *Coefficient of correlation between number of bundles in central region of hypocotyl and central region of epicotyl*

Line	Trimerous			Dimerous		
	N	*r*	r/E_r	*N*	*r*	r/E_r
75.....	416	+.012±.033	0.36	416	−.017±.033	0.52
93.....	557	+.075±.028	2.68	557	+.162±.028	5.79
98.....	345	+.090±.036	2.50	345	+.225±.035	6.43
139.....	106	−.061±.065	0.94	305	−.187±.037	5.05
143.....	143	+.256±.042	6.10	420	+.107±.033	3.24

The correlations are positive with the exception of that for dimerous plants of line 75 and of that for both dimerous and trimerous plants of line 139, which are negative in sign. Only one of the negative coefficients may be considered statistically significant in comparison with its probable error. Several of the positive coefficients are large enough in comparison with their probable errors to be considered possibly significant. The average correlation for the trimerous plants is +.074 while that for the dimerous plants is +.058. The correlations for the trimerous and dimerous plants can not be considered to differ significantly.

The generally positive sign of the constants suggests that seedlings which have a larger number of bundles in the hypocotyl have on the average a larger number of bundles in the epicotyl. This is the condition actually found in the series studied, but the difficulties in the interpretation of the probable error in cases in which the correlation coefficient is so small should make one cautious in generalizing the results obtained.

How slight the relationship between the numbers of bundles in the two organs is, may be shown by the regression lines giving the change in the mean number of bundles in the epicotyl associated with variations in the number of bundles in the hypocotyl and in the mean number of bundles in the hypocotyl associated with variations in the epicotyl. The straight line equations are as follows:

	Dimerous	Trimerous
Line 75,	$H = 10.325 - .068E$	$H = 12.055 + .009E$
	$E = 12.347 - .008H$	$E = 15.267 + .016H$
Line 93,	$H = 5.736 + .401E$	$H = 11.501 + .050E$
	$E = 11.494 + .065H$	$E = 14.273 + .112H$
Line 98,	$H = 1.374 + .648E$	$H = 11.408 + .042E$
	$E = 11.388 + .078H$	$E = 12.538 + .195H$
Line 139,	$H = 4.105 + .338E$	$H = 12.492 - .033E$
	$E = 11.254 + .103H$	$E = 16.591 - .113H$
Line 143,	$H = 6.677 + .161E$	$H = 9.279 + .187E$
	$E = 11.737 + .072H$	$E = 11.810 + .349H$

All of these lines have been drawn, but it seems unnecessary to publish more than three sets.

The comparison between the empirical and the theoretical mean number of bundles in the epicotyls of seedlings classified according to the number of bundles in the hypocotyl is made for three lines on diagram 7. Conversely, the comparison of the actual mean number of bundles in the hypocotyl for plants with various numbers of bundles in the epicotyl is made on diagram 8.

The slight slope of the lines and the irregularity of the empirical means show in a very convincing manner the laxness of the relationship between the numbers of bundles in the central regions of hypocotyl and epicotyl.

These results are of decided morphological significance. The profound difference between the correlations for the hypocotyl and for the epicotyl emphasizes the completeness of the loss of individuality of the bundles at the cotyledonary node. Whereas the number of bundles in the central region of the hypocotyl is quite closely correlated with the number at the base of the hypocotyl, there cannot be asserted to be any significant correlation in bundle number between either the base or the central region of the hypocotyl and the central region of the epicotyl, when we deal with seedlings of the same gross morphological structure. In other words, the reorganization of the vascular system at the node is so complete that the portion of the system which is above the node shows practically no relation to the portion which is below the node.

Comparison of Correlation in Trimerous and Dimerous Seedlings.

In examining the results of the preceding tables the reader may have noted that the coefficients for the dimerous are preponderantly higher than those for the trimerous plants. This result is clearly brought out in table 6

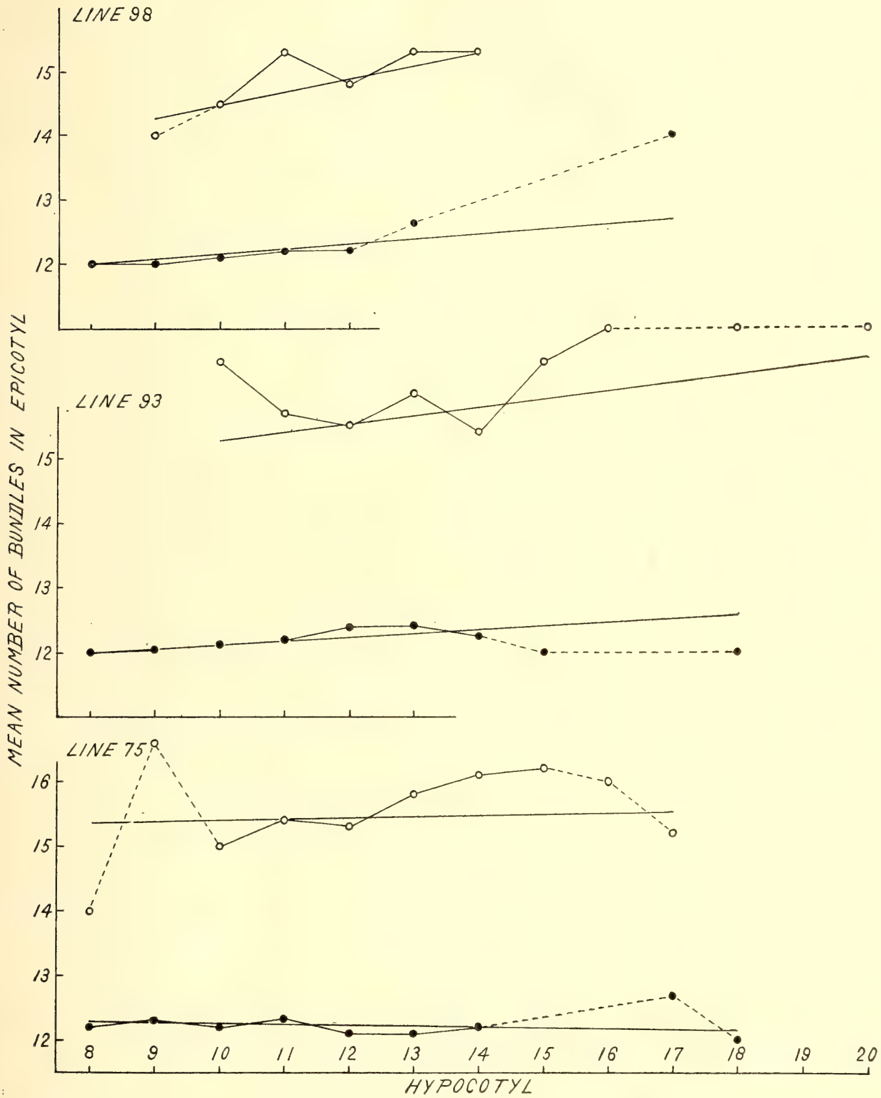


Diagram 7. Regression of number of bundles in central region of epicotyl on number of bundles in central region of hypocotyl. Empirical means represented by solid dots for dimerous seedlings and by circles for trimerous seedlings.

in which the differences between the coefficients for the two classes of plants are shown.

The differences in this table are generally negative, thus indicating that the correlations are lower in the trimerous than in the dimerous seedlings. The exceptions are of some interest.

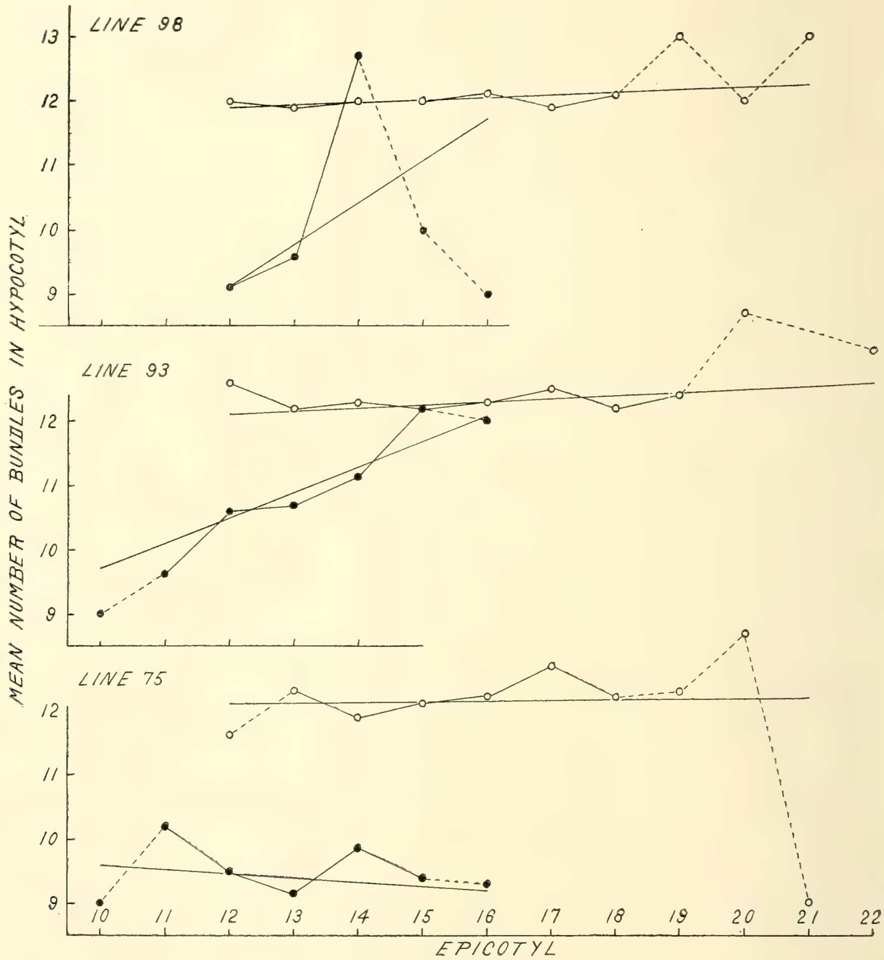


DIAGRAM 8. Regression of number of bundles in central region of hypocotyl on number of bundles in central region of epicotyl. Empirical means represented by solid dots for dimerous seedlings and by circles for trimerous seedlings.

There are only 4 exceptions among the 15 correlations between the numbers of vascular elements in the basal region of the hypocotyl and in the central region of the hypocotyl, as shown in the upper section of the table. These are without exception insignificant in comparison with their probable errors. There are 9 exceptions among the 20 correlations be-

TABLE 6. *Comparison of correlations for trimerous and dimerous seedlings. Differences only (trimerous less dimerous) are given. See tables* 1 *and* 3 *for constants*

	Correlation Coefficient Compared					
	r_{ph}		r_{ih}		r_{bh}	
Line 75..........	+.016±.069	0.23	−.339±.060	5.65	−.148±.039	3.79
Line 93..........	+.408±.060	6.80	−.186±.069	2.69	−.284±.048	5.92
Line 98..........	−.345±.053	6.50	−.302±.058	5.21	−.200±.039	5.13
Line 139..........	+.073±.064	1.14	−.801±.066	12.1	−.394±.047	8.38
Line 143..........	+.026±.039	0.67	−.329±.046	7.15	−.049±.022	2.23
	r_{pe}		r_{ie}		r_{be}	
Line 75..........	+.168±.075	2.24	+.169±.080	2.11	+.236±.079	2.99
Line 93..........	−.171±.076	2.25	−.187±.075	2.49	−.315±.075	4.20
Line 98..........	−.231±.069	3.35	−.039±.070	5.57	−.106±.069	1.53
Line 139..........	−.269±.074	3.64	−.129±.075	1.72	−.270±.075	3.60
Line 143..........	+.006±.056	0.11	+.099±.054	1.83	+.069±.055	1.25
	r_{he}					
Line 75..........	+.029±.047	0.62	—	—	—	—
Line 93..........	−.087±.040	2.18	—	—	—	—
Line 98..........	−.135±.050	2.70	—	—	—	—
Line 139..........	+.126±.075	1.68	—	—	—	—
Line 143..........	+.149±.053	2.81	—	—	—	—

tween the numbers of vascular elements on different sides of the cotyledonary node as shown in the central and lower section. The exceptions occur, in short, among the relationships which in both types of seedlings are practically zero in intensity.

We have no explanation to offer of this greater intensity of correlation in the sub-cotyledonary region of the normal seedling. The result is stated as one of the matters of fact demonstrated by the investigation.

Correlation between Bundle Number in Siblings

The question will naturally arise as to whether the variability in number of bundles in both hypocotyl and epicotyl and the correlation between bundle number in these two internodes may be due to a differentiation of the parent plants from which the seeds were obtained, either in their genetic composition or because of environmental influences. This problem presents many difficulties. Some light may be thrown upon it in the following manner.

An abnormal and a normal seedling were taken from the same parent plant. Thus it is possible to determine in our series the correlation between the number of bundles in the hypocotyl of an abnormal plant and in the hypocotyl of a normal plant derived from the same parent. If a differentiation of the parent plants due to either genetic or physiological factors is the underlying proximate cause of the variability and correlation in bundle number in seedlings which we have studied, there should be a correlation between the number of bundles in the seedlings derived from the same plant.

The correlations between the numbers of bundles in the hypocotyls and epicotyls of the normal and abnormal seedling, *i.e.*, of dimerous and trimerous seedlings, from the same parent plants are given in table 7.[6]

TABLE 7. *Correlations between bundle number in offspring of same parent plant*

Character of Plant and Organs Compared		Line and Correlation		
Trimerous	Dimerous	Line 75	Line 93	Line 98
Hypocotyl	Hypocotyl			
	C. S. H.....	+.0540±.0406	+.1703±.0327	−.0512±.0529
	Storrs......	+.2151±.0540	+.0553±.0540	+.0853±.0495
Epicotyl.......	Epicotyl			
	C. S. H.....	−.0037±.0407	−.0027±.0336	+.1222±.0522
	Storrs......	+.0685±.0563	+.0432±.0541	+.0401±.0498

The coefficients are low throughout. Nine of the 12 are positive while 3 are negative in sign. Only 2 of the 12 can be reasonably regarded as significant. Both of these are positive. There is, therefore, a suggestion of a positive correlation between the anatomical characters of seedlings from the same parent. The values are too low, however, to justify the conclusion that there is a measurable differentiation in the genetic or physiological characteristics of the parent plants affecting bundle number in the offspring seedling.

The absence of correlation here connotes an absence of (sororal or fraternal) inheritance in bundle number.

SUMMARY

In an earlier paper we have shown that the number of vascular elements at different levels in the seedling of *Phaseolus vulgaris* is subject to considerable variation and that the amount of variation may itself differ from level to level. This is true both in normal seedlings with two cotyledons and two primordial leaves and in variant seedlings with three cotyledons and a whorl of three primordial leaves. These two types of seedlings are profoundly differentiated in vascular anatomy as well as in superficial structure.

The purpose of the present paper is to consider the correlations between the number of bundles in the various regions of the seedling. The characters considered are (1) number of primary double bundles, of intercalary bundles, and of total bundles at the *base* of the hypocotyl, (2) number of bundles in the *central region* of the hypocotyl, and (3) number of bundles in the central region of the epicotyl.

1. There is a substantial correlation between each of the three classes of bundles at the base of the hypocotyl and the number of bundles in the central region of the hypocotyl. In the normal seedlings the coefficients

---

[6] It has not seemed worth while to publish the tables upon which these very slight correlations are based. For purposes of comparison the series sectioned at Cold Spring Harbor and at Storrs are both given.

average +.509 for primary double bundles and hypocotyledonary bundles, +.629 for intercalary bundles and hypocotyledonary bundles, and +.813 for total bundles and hypocotyledonary bundles. In the trimerous plants these correlations average +.381, +.238, and +.598, respectively.

The correlations for normal plants are generally higher than those for abnormal plants.

2. The correlation between each of the three classes of bundles at the base of the hypocotyl and the number of bundles in the central region of the epicotyl is low. The coefficients are sometimes positive and sometimes negative in sign. On the basis of the data available it is impossible to assert that there is any correlation at all between the numbers of bundles in these two regions.

3. The correlation between the numbers of bundles in the central region of the hypocotyl and in the central region of the epicotyl is likewise very low. The coefficients are generally not significant in comparison with their probable errors. If there be any correlation at all between the numbers of bundles in these two regions it is very slight indeed.

These results for correlation fully substantiate the conclusions drawn in an earlier paper that there is a *complete reorganization of the vascular system at the cotyledonary node.*

4. The correlation between the number of bundles (either hypocotyledonary or epicotyledonary) in siblings is, if it exists at all, very low. The differentiation of the parent plants through either genetic or environmental factors cannot, therefore, be considered to be the source of the variation and correlation in bundle number demonstrated in this and in our preceding paper.

Conclusions

These results, and others for which the reader must turn back to the body of the paper, justify the emphasis at this point of the following general conclusions:

a. The vascular structures of the seedling are not constant but are decidedly variable within the species. They show different degrees of variability within the individual organism.

b. Seedlings differing in external form are profoundly differentiated in their internal anatomy. This differentiation is evident both in mean number of bundles and in the degree of variability in bundle number. In short, the external form and the internal structure of the seedling are highly but not perfectly correlated.

c. The different anatomical characters of the seedling are interrelated with varying degrees of intensity. Between some there is a very strong correlation, but between others practically none at all.

The quantitative measurement and interpretation of such relationships, by means of the biometric methods hitherto little applied in the field of vascular morphology, will make possible material advance in the investigation of the fundamental problems of morphogenesis.

TABLE A. *Data for correlation between bundle number at the base of the hypocotyl and in the central regions of hypocotyl and epicotyl in trimerous seedlings*

Base*	Line	Hypocotyl													Epicotyl											Totals
		8	9	10	11	12	13	14	15	16	17	18	19	20	12	13	14	15	16	17	18	19	20	21	22	
(4)+0...	143	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	1	..	..	..	..	2
(4)+1...	139	..	..	1	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	1
	143	..	1	..	1	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	3	..	..	..	..	3
(4)+4...	93	..	..	..	..	..	1	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	1
(4)+5...	98	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	1
(4)+6...	75	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	1
(5)+0...	75	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	1
	93	..	..	1	1	3	..	..	..	..	..	..	..	..	..	..	..	1	1	2	..	1	..	..	..	5
	98	..	..	3	..	1	..	..	..	..	..	..	..	..	..	..	1	3	..	..	..	..	..	..	..	4
	139	..	..	1	2	1	..	..	..	..	..	..	..	..	..	1	..	1	..	1	..	1	..	..	..	4
	143	..	..	11	2	1	..	..	..	1	..	..	..	..	2	..	3	3	3	1	..	3	..	..	..	15
(5)+1...	75	..	..	2	2	3	1	..	..	..	..	..	..	..	..	..	2	3	1	2	..	..	..	..	..	8
	93	..	..	..	6	3	..	1	..	..	..	..	..	..	1	..	1	1	3	1	2	1	..	..	..	10
	98	..	..	1	3	2	..	..	..	..	..	..	..	..	..	1	2	2	1	..	..	..	..	..	..	6
	139	..	..	1	1	1	1	..	..	..	..	..	..	..	..	..	..	1	1	..	2	..	..	..	..	4
	143	..	..	..	11	13	2	4	..	..	1	..	..	..	..	2	2	9	6	5	3	1	2	1	..	31
(5)+2...	75	..	..	..	..	..	1	..	..	..	1	..	..	..	..	..	1	..	1	..	..	..	..	..	..	2
	93	..	..	..	..	..	1	2	..	..	..	..	..	..	..	..	1	..	..	1	1	..	..	..	..	3
	98	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	1
(6)+0...	75	..	..	..	6	90	8	3	..	..	..	..	..	..	..	5	19	43	22	11	6	..	1	..	..	107
	93	..	..	..	2	102	10	4	1	..	..	..	..	1	..	6	14	47	25	15	8	3	1	..	1	120
	98	..	..	..	4	145	8	3	..	..	..	..	..	..	6	13	36	67	26	8	3	..	1	..	..	160
	139	..	..	1	5	81	3	1	1	..	..	..	..	..	..	4	21	35	21	8	2	1	..	..	..	92
	143	..	..	..	..	121	7	4	1	1	..	..	..	..	3	7	14	34	31	22	19	3	1	..	..	134
(6)+1...	75	..	..	..	..	1	9	2	..	..	..	..	..	..	..	..	..	6	3	1	..	1	1	..	..	12
	93	..	..	..	..	..	8	3	..	..	..	..	..	..	1	..	..	6	2	1	1	..	..	..	..	11
	98	..	..	..	..	..	7	3	..	..	..	..	..	..	..	..	3	3	2	..	1	1	..	..	..	10
	139	..	..	..	..	1	2	2	..	..	..	..	..	..	..	2	..	1	2	..	..	..	..	..	..	5
	143	..	..	..	..	1	11	12	1	..	..	..	..	..	..	..	..	5	5	8	3	2	2	..	..	25
(6)+2...	75	..	..	..	..	1	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	2	..	..	2
	93	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	1
(7)+0...	75	..	..	..	..	..	..	7	..	..	..	..	..	..	..	..	..	4	2	1	..	..	..	..	..	7
	93	..	..	..	..	..	..	3	1	..	..	..	..	..	..	..	2	..	1	1	..	..	..	..	..	4
	98	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	1
	143	..	..	..	..	..	..	5	..	..	..	..	..	..	..	..	..	2	2	1	..	..	..	..	..	5
(7)+1...	143	..	..	..	..	..	..	..	3	1	..	..	..	..	..	..	..	1	1	..	1	..	..	1	..	4
(7)+2...	75	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	1
(8)+0...	75	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	1	..	..	..	..	..	1
	143	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	1
(8)+1...	143	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	1	..	..	1

* Numbers in parentheses are of *primary double bundles*; those following are of *intercalary bundles*.

Table B. *Data for correlation between bundle number at the base of the hypocotyl and in the central regions of hypocotyl and epicotyl in dimerous seedlings*

Base	Line	Hypocotyl										Epicotyl							
		8	9	10	11	12	13	14	15	17	18	10	11	12	13	14	15	16	Tot.
(4)+0 . . .	75	40	17	7	4			1				1	1	59	4		2	2	69
	93	12	13	6	3									29	4	1			34
	98	57	31	9										88	8			1	97
	139	269	1											250	18	2			270
	143	262	21	6	2									229	43	13	4	2	291
(4)+1 . . .	75		14	13	3								2	22	3	2		1	30
	93		14	17	3	1	1	1						31	3	3			37
	98	2	26	13	1	1								37	5	1			43
	139		22	3	1									21	4	1			26
	143	1	61	27	10		2		1					72	17	11	1	1	102
(4)+2 . . .	75			2	4	3	1							9	1				10
	93			9	2	2								11	2				13
	98		3	13	6	1								19	3		1		23
	139			2	2	1	1							5	1				6
	143			3	1	1								3		1		1	5
(4)+3 . . .	75				2	1					1			4					4
	93				3	2								3		1	1		5
	98				2									2					2
(4)+4 . . .	75							1		1				2					2
	93					1								1					1
(4)+5 . . .	75						1			1				2					2
(5)+0 . . .	75		1	8	2	1	1							12	1				13
	93		1	13	4	3	1							18	1	2	1		22
	98			2	3	1								4	1	1			6
	139			1										1					1
	143		1	11		1								8	5				13
(5)+1 . . .	75				2	2								4					4
	93			1	6	7	3	1						12	3	1	2		18
	98			1	5	1	1							7	1				8
	139			1	1									1		1			2
	143				4	2	1							4	1	2			7
(5)+2 . . .	75					1								1					1
	93					5	3	1						6	1	2			9
	98					1								1					1
(5)+3 . . .	75							1						1					1
	93							1						1					1
	98						1									1			1
(6)+0 . . .	75				1	3		1					1	4					5
	93				1	8	1							9	1				10
	98				1									1					1
(6)+1 . . .	75					1								1					1
	93						1	2						2	1				3
	143								1					1					1
(6)+2 . . .	93							2						1		1			2
(7)+0 . . .	143							1						1					1
(8+1) . . .	98									1						1			

Table C. *Correlation between numbers of bundles in hypocotyl and epicotyl of trimerous plants of line 75*

Hypocotyl	Epicotyl										Totals
	12	13	14	15	16	17	18	19	20	21	
8	...	...	1	...	...	...	...	...	...	...	1
9	...	...	1	1	...	...	...	...	...	1	3
10	...	...	...	5	...	...	...	...	...	...	5
11	1	...	6	12	12	3	2	...	...	...	36
12	2	15	48	120	62	21	19	3	2	...	292
13	...	...	5	13	10	7	4	1	...	...	40
14	...	...	1	10	7	8	2	...	1	...	29
15	...	...	1	2	...	1	...	...	1	...	5
16	...	...	...	...	1	...	...	...	...	...	1
17	...	1	...	1	1	1	...	...	...	...	4
Totals	3	16	63	164	93	41	27	4	4	1	416

Table D. *Correlation between numbers of bundles in hypocotyl and epicotyl of dimerous plants of line 75*

Hypocotyl	Epicotyl							Totals
	10	11	12	13	14	15	16	
8	...	...	116	17	6	4	...	143
9	1	2	78	13	5	2	2	103
10	...	...	74	10	...	1	1	86
11	...	1	29	4	2	2	...	38
12	...	1	22	1	1	1	...	26
13	...	...	6	1	...	...	...	7
14	...	...	8	...	1	...	...	9
15	...	...	...	...	...	...	...	...
16	...	...	...	...	...	...	...	...
17	...	...	2	...	1	...	...	3
18	...	...	1	...	...	...	...	1
Totals	1	4	336	46	16	10	3	416

Table E. *Correlation between numbers of bundles in hypocotyl and epicotyl of trimerous plants of line 93*

Hypocotyl	Epicotyl											Totals
	12	13	14	15	16	17	18	19	20	21	22	
10	...	...	...	3	1	1	3	...	...	...	...	8
11	...	...	3	14	7	4	3	1	...	...	...	32
12	3	14	33	170	92	38	26	5	1	...	...	382
13	1	4	5	30	15	6	16	3	1	...	1	82
14	1	...	5	17	9	2	3	1	...	...	...	38
15	...	...	1	2	5	2	...	...	2	...	...	12
16	...	...	...	...	...	1	...	...	...	...	...	1
17	...	...	...	...	...	...	...	...	...	...	...	...
18	...	...	...	...	...	1	...	...	...	...	...	1
19	...	...	...	...	...	...	...	...	...	...	...	...
20	...	...	...	...	...	1	...	...	...	...	...	1
Totals	5	18	47	236	129	56	51	10	4	...	1	557

TABLE F. *Correlation between numbers of bundles in hypocotyl and epicotyl of dimerous plants of line 93*

Hypocotyl	Epicotyl							
	10	11	12	13	14	15	16	Totals
8		1	31	2				34
9	1	1	85	3	3			93
10		3	147	14	3	2		169
11		1	88	14	2			105
12			78	5	9	3	1	96
13			31	4		4		39
14			16		1	1		18
15			1					1
18			2					2
Totals	1	6	479	42	18	10	1	557

TABLE G. *Correlation between numbers of bundles in hypocotyl and epicotyl of trimerous plants of line 98*

Hypocotyl	Epicotyl										
	12	13	14	15	16	17	18	19	20	21	Totals
9			1								1
10		1	1	4							6
11		1	2	4	3	1	1				12
12	8	20	58	157	41	8	4		1		297
13		2	6	8	1		2	1		1	21
14			1	3	4						8
Totals	8	24	69	176	49	9	7	1	1	1	345

TABLE H. *Correlation between numbers of bundles in hypocotyl and epicotyl of dimerous plants of line 98*

Hypocotyl	Epicotyl					Totals
	12	13	14	15	16	
8	107	6				113
9	103	6			1	110
10	71	4	1	1		77
11	26	5	1			32
12	7	2				9
13	2		1			3
17			1			1
Totals	316	23	4	1	1	345

Table I. *Correlation between numbers of bundles in hypocotyl and epicotyl of trimerous plants of line 139*

Hypocotyl	Epicotyl							
	13	14	15	16	17	18	19	Totals
10	2				1	1		4
11	1	1	2	1	2		1	8
12	4	19	32	20	5	3	1	84
13		1	2	2	1			6
14	1		2					3
15				1				1
Totals	8	21	38	24	9	4	2	106

Table J. *Correlation between numbers of bundles in hypocotyl and epicotyl of dimerous plants of line 139*

Hypocotyl	Epicotyl			Totals
	12	13	14	
8	249	18	2	269
9	20	3		23
10	5	1	1	7
11	2	1	1	4
12	1			1
13	1			1
Totals	278	23	4	305

Table K. *Correlation between numbers of bundles in hypocotyl and epicotyl of trimerous plants of line 143*

Hypocotyl	Epicotyl										
	12	13	14	15	16	17	18	19	20	21	Totals
8					1		1				2
9							1				1
10	2		2	2	2	1		2			11
11			2	2	5	2	1	1	1		14
12	3	9	14	35	31	22	19	3			136
13			1	6	5	5	2	1	1		21
14				7	4	6	4	1	2	1	25
15				2			3			1	6
16					1			1	1		3
17						1					1
18									1		1
Totals	5	9	19	54	49	37	31	9	6	2	221

TABLE L. *Correlation between numbers of bundles in hypocotyl and epicotyl of dimerous plants of line 143*

Hypocotyl	Epicotyl					Totals
	12	13	14	15	16	
8	205	42	10	4	2	263
9	65	11	7			83
10	32	8	5		2	47
11	9	3	4	1		17
12	3		1			4
13	1	2				3
14	1					1
15	2					2
Totals	318	66	27	5	4	420

A QUANTITATIVE STUDY OF THE EFFECT OF ANIONS ON THE PERMEABILITY OF PLANT CELLS. II

Oran L. Raber

(Received for publication January 20, 1921)

Studies on the effect of certain anions upon permeability were reported in a previous paper (1). The present paper extends the list of anions, including both organic and inorganic. The field is very limited because of the restrictions of solubility, osmotic pressure, acidity, etc., which were mentioned in a previous paper. The sodium salts which seemed to offer the fewest possible difficulties were chlorate, dichromate, molybdate, sulphite, acid arsenate, ferrocyanide, formate, propionate, lactate, butyrate, and salicylate. The following table shows the acidity and concentrations of the solutions:

Salt	Approximate pH	Approximate Mol. Conc.
$NaClO_3$	6	0.61
$Na_2SO_3.7H_2O$	11	0.35
$Na_2Cr_2O_7.2H_2O$	3	0.29
$Na_2MoO_4.2H_2O$	6	0.39
$Na_2HAsO_4.7H_2O$	8	0.34
$Na_4Fe(CN)_6.12H_2O$	8	0.13
$NaCHO_2.H_2O$ (formate)	10	0.56
$NaC_3H_5O_3$ (lactate)	6	1.61
$NaC_4H_7O_2$ (butyrate)	10	1.70
$NaC_3H_5O_2$ (propionate)	9	1.74
$NaC_7H_5O_3$ (salicylate)	6	1.29

With one exception the pH values lie within the limits which Osterhout (2) has shown to have no effect upon permeability (in sea water). In every case, with the exception of the ferrocyanide and the molybdate, the conductivity was that of normal sea water. In both the latter cases the conductivity was approximately that of 75 percent sea water plus 25 percent distilled water.

Figure 1 shows the results, the curve for each salt representing an average of three experiments. The probable error of the mean (as based on Peter's formula) is always under 10 percent of the mean (and for 75 percent of the points under 5 percent).

It will be noted that the univalent anions (formate and chlorate), the bivalent (molybdate and sulphite), and the trivalent (arsenate) fall into three separate groups, as in the case of the anions previously reported (1).

It might be expected that the ferrocyanide would cause a very rapid decrease in resistance since it is a tetravalent ion, and its failure to do so

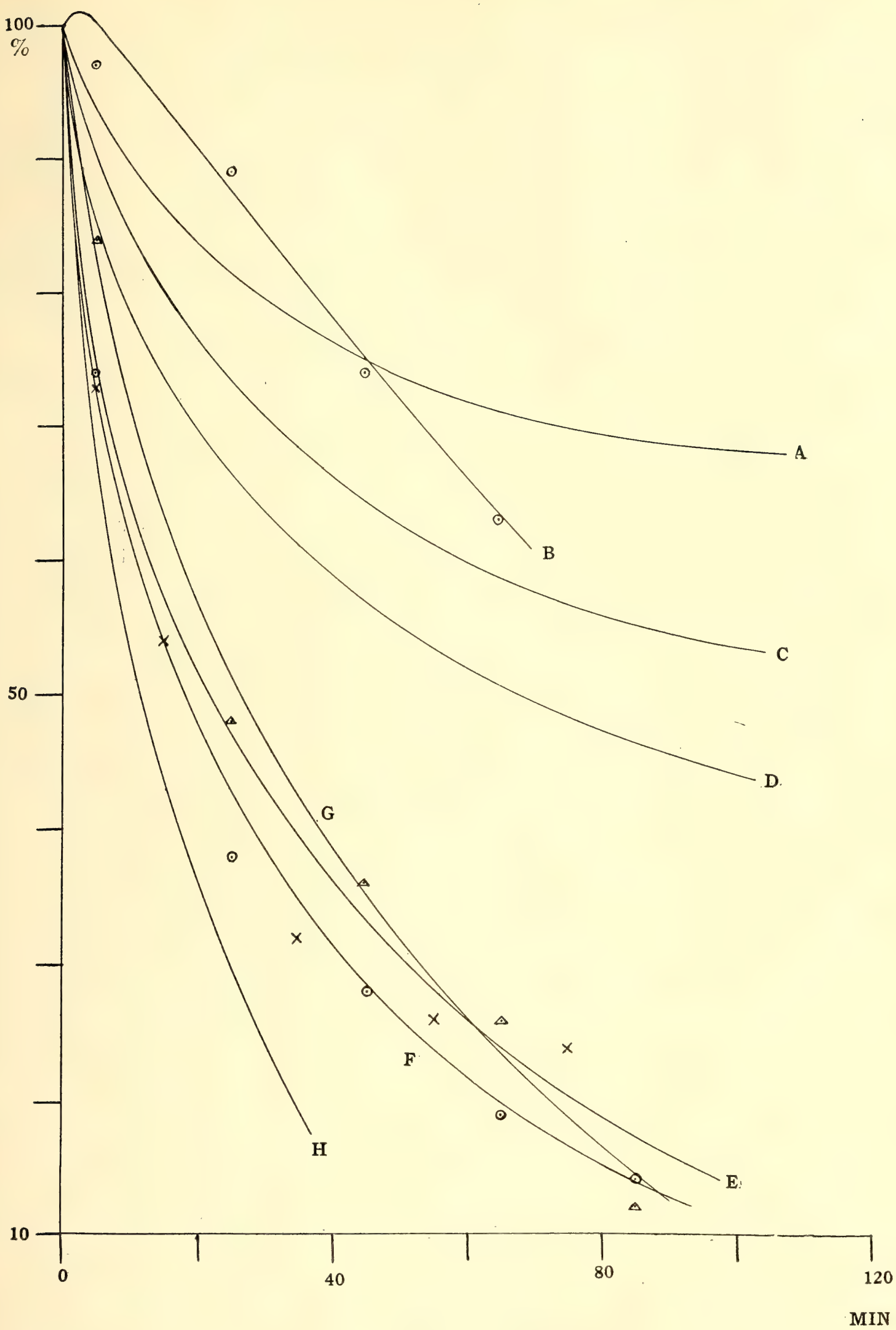


FIG. 1. Curves showing the changes of resistance of Laminaria in solutions of various sodium salts: *A* in chloride; *B* in dichromate; *C* in chlorate; *D* in formate; *E* in molybdate (to judge from the points in the figure this might be drawn so as to fall less rapidly toward the end, but the form given seems to represent the results better); *F* in sulphite; *G* in ferrocyanide; *H* in acid arsenate. Ordinates represent resistance (expressed as percentage of the resistance in sea water, which is taken as 100 percent). Abscissae represent time in minutes. Each point represents the average of three experiments. Probable error of the mean, less than 10 percent of the mean.

may be explained on the basis of its small molecular concentration (0.13 M). If all these salts were of the same molecular strength, they would doubtless be found to fall into four groups depending on their valency.

The sodium dichromate forms an exception because of its acidity. The acidity is slightly higher than the limit which was shown by Osterhout (2) to be without effect on permeability, with the result that there is a very small initial increase followed by a slow but steady decrease. This indicates antagonism between the hydrogen ion and the anion.

It is also interesting to note that, although arsenic is usually regarded as a very toxic substance, the effect of the arsenate does not seem to be any greater than that of any other salt with trivalent anion.

Among the organic salts only formate gave useful results. The osmotic effects produced by the high concentrations necessary in the cases of the propionate, lactate, butyrate, and salicylate cause a very sudden fall of resistance. The effect upon the tissue, however, is very different from that produced by a salt like sodium citrate. In the former cases the tissue simply becomes flabby without undergoing any other noticeable change. In the case of sodium citrate (and similar salts), where the rapid fall is not due to plasmolysis, the tissue becomes decidedly gelatinous and presents a very different appearance.

The results indicate that the effects upon permeability depend upon the valency of the anion regardless of whether the salts are organic or inorganic.

LABORATORY OF PLANT PHYSIOLOGY,
HARVARD UNIVERSITY

BIBLIOGRAPHY

1. **Raber, O. L.** A quantitative study of the effect of anions on the permeability of plant cells. Jour. Gen. Physiol. 2: 535. 1920.
2. **Osterhout, W. J. V.** The effect of acid on permeability. Jour. Biol. Chem. **19**: 493. 1914.

THE MECHANISM OF ROOT PRESSURE AND ITS RELATION TO SAP FLOW[1]

James Bertram Overton

(Received for publication January 21, 1921)

The important rôle played by osmotic pressure in plants is well recognized. Plant physiologists assume that osmotic pressure in the peripheral cells of the root is in some way connected with the process by which sap is continuously supplied to the xylem vessels, and the complex conditions controlling the movement of water from the soil through the root to the vessels are passed over by the use of the phrase "root pressure." Most of us are familiar with the contributions of Dixon, supported by the rather brilliant work of Renner, that water passes through the roots to the leaves because the leaves tend to dry out due to water being abstracted from them by evaporation, resulting in the water in the vessels passing into a state of tension, which is transmitted equally in all directions. This condition obtains unless root pressure or atmospheric pressure or both is forcing water up the stem more rapidly than it evaporates.

The cohesion theory of sap flow has received much attention and has been supported by considerable evidence. Transpiration or growth appears to produce an increase in the "saturation deficit" (Renner) or "incipient drying" (Livingston) in the exposed leaf cell walls which is followed by a corresponding increase in the saturation deficit in all the cells abutting upon the intercellular spaces of the leaf. Dixon points out that this condition is a sufficient cause for the entrance of water into the root and its passage through the root periphery. Renner has shown that the water-absorbing power of the root is directly related to the saturation deficit in the leaves above, and that the root's absorbing power appears to be directly referable to the tendency of the exposed cell walls of the root to dry out on account of passage of water to other parts of the plant. According to his view, during transpiration the root is rendered flaccid and therefore able to absorb water, although root pressure and passive absorption may under certain conditions work in combination.

Dixon holds that the entry of water into the root depends upon the gradient of pressure as we pass from the outside of the root to the inside of the tracheae, there being a fall of pressure due to the continuous water all the way up the stem to the leaves; thus the flow of water up the highest trees is due to the evaporation and condensation produced by the difference between the vapor pressure in the soil spaces and that obtaining around the leaves, and the tensile water flows under the action of this difference from end to end of the plant. The tensile stress transmitted to the root has the ultimate effect of drying out the root surface, and the gradient of pressure, which causes the passage of water from the soil into the root, is referable to the concave menisci formed in the cell walls of the root periphery due to the tension in the sap.

---

[1] Invitation paper read before the Physiological Section of the Botanical Society of America, in the symposium on biophysics, at Chicago, December 28, 1920.

According to the above-outlined picture it would appear that in the absence of root pressure the passage of water from the soil into the root periphery is purely passive, the root acting merely as a filter in water intake, a view, however, to which Jost raises objections, although it has been shown that much water may be taken in by dead roots and that roots of transpiring plants can absorb water from solutions of high osmotic pressures. Concerning the mechanics of root absorption, however, we know comparatively little on account of the difficulties of observation, and little is actually known about the forces which are operative in the passage of water from the soil through the root into the vessels in sufficient quantity to supply the transpiration needs.

Root pressure is not generally considered as important in causing the ascent of sap through the stem. The presence of a negative gas pressure in the vessels shows that the water is not being forced up from below, and root pressure is lowest when transpiration is most active and highest when water movement in the stem is slowest. Dixon regards the function of root pressure to be a periodic flooding of the vessels with water, tending to bring the gas bubbles into solution and to reëstablish conditions for tension throughout the water tracts, the influence of root pressure in sap flow being an indirect one. The importance of osmotic phenomena in the root as far as water is concerned remains to be determined. It has been suggested by Livingston and Pulling that root pressure is mainly effective in maintaining the form of the roots and their contact with the water films of the surrounding soil. It has been shown that the rate of water absorption by roots is not proportional to the osmotic pressure of the root cells and that they can operate equally well with high or low turgidity so long as they are not deformed.

The exudation of liquid water from passive hydathodes, such as in Colocasia, and the secretion of water and solutes from wounds, show that the roots of many plants under certain conditions can develop considerable pressure. Whether or not it is admitted that sap pressure functions in sap flow, it seems certain that root cells are able to take in water and solutes and to pass them on to the xylem. Atkins holds that the cortical cells of the root have a much higher osmotic pressure than the tracheae and that they function as a complex semi-permeable membrane. It is evident that sap pressure, whether in roots or elsewhere, depends upon osmotic phenomena. It would appear from a survey of the literature that sap movement in roots is to be explained in the same way as the action of glands and hydathodes, and that there exists the unilateral secretion of water and solutes from the root parenchyma into the vessels, which is an osmotic phenomenon. The question arises as to how such a unilateral excretion can take place from a turgescent cell.

Pfeffer first pointed out the necessity of investigating experimentally the secretion of water and solutes in one-celled plants as a basis for understanding the phenomena in hydathodes. This Lepeschkin has done, and on the basis of experiments he explains the continuous secretion of water and solutes by Pilobolus by assuming a different semi-permeability on the upper and lower sides of the cell. He finds that the lower or absorbing part of the sporangiophore may possess a greater osmotic pressure than the upper or secreting portion, and adopts Pfeffer's assumption that, if the cell sap at different points in the cell has different concentrations, the inflow must exceed the outflow until an equality is reached between the sides, so that there results a unilateral exudation of water through the upper, more permeable, membrane, brought about by a pressure corresponding to the difference in concentration on the two sides of the cell. The same holds true for the epidermal secreting structures of phanerogams and ferns.

Pfeffer's scheme assumes an unequal distribution of osmotic material in the protoplasm, the concentration being greatest in that part of the cell which is richest in osmotic substances. Lepeschkin has actually determined that such a condition obtains in Pilobolus, which indicates in this case that the two opposite ends of the cell are chemically and physically different. We should, therefore, expect that the water-absorbing and the water-holding capacity of the two sides of the cell would be different. Copeland actually constructed a piece of apparatus by which a current of water is maintained through an artificial cell by using membranes unequally permeable, thus proving Pfeffer's assumption, and holds that it is possible for the osmotically active substances in a cell to exert different pressures in different directions if the protoplasm is permeable to these substances in different degrees in different parts. Copeland points out that root pressure must be due to the same process, and that in order for root pressure to be caused in this way the protoplasm must be permeable to the osmotically active matter of the cell sap to a different degree in different parts of itself.

On the basis of Lepeschkin's results and of some of his own, Priestly recently attempts to explain the movement of water and solutes through the root and their excretion into the xylem vessels. He points out, as has been suggested by Atkins, that on account of sugar being brought down from the leaves by the vascular elements, the sap of the cells bordering on the vessels will tend to be more concentrated than that of the cells further out, and an osmotic gradient will be established from these cells through the root parenchyma to the root periphery. As a result of this osmotic gradient, water will enter at the periphery and pass toward the center, gradually distending the cells of the parenchyma of the vascular cylinder.

The parenchyma within the endodermis, being confined, is limited in extensibility on account of the structure of the endodermal cells. A strong hydrostatic pressure will therefore develop in this core of cells, sufficient to cause an excretion of water and solutes into the xylem vessels so long as the osmotic gradient obtains. When the water and solutes enter the xylem they are free to move upward in the vessels. Water may leak backward as far as the endodermis but no further on account of the suberized walls of the endodermis, which structure has been shown to prevent such a backward leakage. Priestly shows that the apical region of the root does not permit a backward leakage.

This explanation of root absorption and secretion offers some difficulties. Priestly points out that it is difficult to understand how the necessary solutes can be provided in sufficient quantities to permit a constant flow of water across the inner membranes of the cells next to the xylem vessels, where a considerable amount of water may pass upward. On the basis of Lepeschkin's results, Priestly suggests that such solutes might be either organic or inorganic, and that in the root they are organic, either sugars or, more probably, organic acids derived from sugars, and cites Atkins' results as to the presence of sugar in the ascending sap. Curtis, however, holds that the xylem does not serve for longitudinal translocation of carbohydrates.

In harmony with Bayliss, Priestly suggests that normal semi-permeability to glucose is a function of the difference in concentration on the two sides of the membrane, so that an accumulation or a dilution of sugar within the cells bordering on the xylem vessels might lead to a change of permeability in these cells and result in rendering the plasma membrane on the inner side temporarily permeable to sugar, and thus in an intermittent excretion into the xylem vessels. As Priestly suggests, the process, although intermittent, would appear as a continuous one in root pressure, due to the combined activity of many cells.

Flood finds that the exudation of water from Colocasia leaves does not depend upon any special secretion tissue in the leaves, but that the phenomenon rests upon the action of cells lower down, probably in the root. During its passage upward from the roots until its exudation, the water passes through no filtration membranes. The water exuded is almost free from solutes as has been shown by Atkins. If we assume that solutes are excreted into the xylem along with the water, the question arises as to what becomes of these solutes in the case of Colocasia. Priestly suggests that they are adsorbed during their passage upward by the protoplasts surrounding the vessels.

Transpiration has by some been looked upon as a function on the supposition that it is useful in concentrating the salts brought to the leaves, a supposition to which certain workers object on the ground that this assumption carries with it the further assumption that water in the vessels carries the solutes along with it. These assumptions are held to contradict theory and observation on osmotic movement. The general conception of the function of the xylem is that, in times when water is abundant, it carries the inorganic substances to the leaves; but Atkins finds that sugars are at all times present in the sap of the vessels, usually in greater quantities than the electrolytes. Therefore, the vessels should be regarded as transferring both water and solutes, organic as well as inorganic. This view, however, is opposed to that of Curtis, who suggests that the interposition of living cells across conducting tubes may prevent a flow of solution, and that water may normally flow largely by diffusion.

It has, however, been shown that with the possible exception of Colocasia secretions contain both electrolytes and non-electrolytes.

An attempt has been made in this discussion to explain the entrance of water and solutes into the vessels in the presence of root pressure. From a physical point of view it is difficult to conceive why solutes should not be carried with the transpiration current once they enter the vessels. If, in the absence of root pressure, and when there exists a negative pressure in the vessels, water enters the root by filtration, it is also conceivable that soil solutes may also enter along with the water unless some mechanism exists in the root to check their entrance. Curtis, however, holds that there is no flow of solution from the soil into the root, and that the amount of water absorbed bears no relation to the amount of solutes absorbed. Pfeffer states that

> A substance imbibed by the cellulose cell walls may reach the center of a tissue without having penetrated a single protoplast. It is indeed possible that the water and salts absorbed by the roots pass mainly, if not entirely, through either the cell walls of living cells or the walls and cavities of dead wood fibers, etc., so that only on reaching the crown of the tree do they penetrate the protoplasts of the actively growing tissues localized there.

If we admit that soil solutes enter the vessels either by filtration or by excretion or both, and are carried to the leaves by the transpiration current, some provision must be conceived to prevent their too great concentration in the leaf cells, otherwise in the course of a transpiring season the leaves would become rigid with salt. Possibly the endodermis may function in controlling the entrance of soil solutes as well as in preventing the backward leakage of water and solutes from the root. Some evidence already exists to support this view, as substances which cannot penetrate the endodermis do not penetrate into the vascular cylinder of the root. In fact, De Rufz de Lavison shows that the suberized walls of endodermal cells are impermeable, that all solutes which enter the vascular cylinder of the root are forced to pass through the protoplasm of the endodermis, and also that cellulose cell walls of the root tip are of such a character that only such solutes enter this region as are able to penetrate the protoplasm. The supposition is, therefore, that the quantity and quality of solutes which penetrate into the root depend upon a sort of filtration across the protoplasm of the endodermis and that of the root tip. It may be that in the absence of root pressure and in the presence of a negative pressure in the vessels, water and solutes may pass through the endodermis and through the root tip in a purely physical manner. Evidence also exists to support the view that certain amounts of salts are concentrated in the leaves, and it is possible that these salts are thrown out of solution by the protoplasm so as not to upset the osmotic relation of the cells. The assumption that solutes are adsorbed by the living protoplasts accompanying the vessels is also suggestive.

Whatever may be the mechanism of the entrance of solutes into the root and of their passage into vessels, it would appear that the problems here stated are worthy of serious and careful investigation.

Department of Botany,
University of Wisconsin

BOTANICAL SOCIETY OF AMERICA

Reorganized 1906, uniting the Botanical Society of America (1893), the Society for Plant Morphology and Physiology (1896), and the American Mycological Society (1903).

OFFICERS FOR 1921

President: C. E. ALLEN
University of Wisconsin

Vice-President: H. C. COWLES
University of Chicago

Secretary: J. R. SCHRAMM
Cornell University, Ithaca, N. Y.

Treasurer: IRVING W. BAILEY
Bussey Institution, Harvard University

Councilors:

N. L. BRITTON
New York Botanical Garden

WILLIAM TRELEASE
University of Illinois

J. C. ARTHUR
Purdue University

Any one actively interested in botanical work is eligible for membership. Candidates for membership are recommended by three members, not members of the Council, on blanks to be obtained from the Secretary.

The annual dues are $5.00, including subscription to the AMERICAN JOURNAL OF BOTANY. Candidates proposed for membership, and approved by the Council, may receive one copy of the JOURNAL at $4.00 per year pending their election at the winter meeting of the Society.

The Little Things
That Count Big
In Owning Your Own
Greenhouse

Ever gone into a hat store, knowing exactly the kind and shaped hat you want; and then have some smart Alec of a salesman try to sell you the kind HE likes?

Makes a fellow madder than a wet hen, doesn't it?

Suppose, however, that same salesman had told you something about some of his hats that meant better service to you—more satisfaction for your money—you then sort of feel towards that salesman as you would an old friend.

Salesman number two is our sort when it comes to selling a greenhouse.

We do sell greenhouses, but we first sell satisfaction.

Catalog for the asking.

Hitchings and Company

Home Office and Factory: ELIZABETH, N. J.

NEW YORK
101 Park Ave.

BOSTON-9
294 Washington St.

otany

Vol. VIII. OCTOBER, 1921 No. 8

AMERICAN JOURNAL OF BOTANY

OFFICIAL PUBLICATION OF THE
BOTANICAL SOCIETY OF AMERICA

Smithsonian Institution
NOV 16 1921
National Museum.

CONTENTS

The vascular anatomy of hemitrimerous seedlings of Phaseolus vulgaris. J. ARTHUR HARRIS, EDMUND W. SINNOTT, JOHN Y. PENNYPACKER AND G. B. DURHAM 375
The effect upon permeability of polyvalent cations in combination with polyvalent anions . . . . ORAN L. RABER 382
The floral anatomy of the Urticales . . . . ALBERT REIFF BECHTEL 386
Genetic evidence of aberrant chromosome behavior in maize endosperm. R. A. EMERSON 411

PUBLISHED
IN COOPERATION WITH THE BOTANICAL SOCIETY OF AMERICA
BY THE
BROOKLYN BOTANIC GARDEN
AT 41 NORTH QUEEN STREET, LANCASTER, PA.

Entered as second-class matter February 21, 1914, at the post office at Lancaster, Pennsylvania, under the act of March 3, 1879

AMERICAN
JOURNAL OF BOTANY

DEVOTED TO ALL BRANCHES OF BOTANICAL SCIENCE

ESTABLISHED 1914

EDITED BY A COMMITTEE OF THE
BOTANICAL SOCIETY OF AMERICA

EDITORIAL COMMITTEE

C. E. ALLEN, Editor-in-Chief,
University of Wisconsin

WILLIAM CROCKER,
The Thompson Institute For Plant Research, Inc.

L. R. JONES,
University of Wisconsin

C. STUART GAGER, Business Manager
Brooklyn Botanic Garden

ARTHUR H. GRAVES
Brooklyn Botanic Garden

R. A. HARPER,
Columbia University

JACOB R. SCHRAMM,
Cornell University

C. L. SHEAR, *Bureau of Plant Industry*
(Representing American Phytopathological Society)

The Journal is published monthly, except during August and September. Subscription price, $6.00 a year. Single copies 75 cents. Back numbers, 75 cents each; $7.00 a volume. Postage will be charged to all foreign countries, except Mexico, Cuba, Porto Rico, Panama Canal Zone, Republic of Panama, Hawaii, Philippine Islands, Guam, Samoan Islands, and Shanghai. Postage to Canada, 20 cents a volume on annual subscriptions; to all other countries in the Postal Union, 40 cents a volume on annual subscriptions.

The pages of the Journal are open to members of the Botanical Society of America, or to candidates approved for membership.

Manuscript offered for publication should be typewritten, and should in all cases be submitted to the Editor-in-Chief.

Papers are limited to 20 pages in length, exclusive of plates, except that additional pages may be arranged for at cost rate to authors (approximately $3.00 a page).

Proofs should be corrected immediately on receipt, and returned to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y.

Illustrations, other than zinc etchings, and tabular matter should, in general, be restricted to approximately ten percent of the number of pages of text. Authors may be charged at cost rates for illustrations and tabular matter in excess of that amount (approximately $6.40 a page for tabular matter; 15 cents per square inch for half-tones).

Separates should be ordered when proof is returned. Ffty copies without cover will be supplied free; cover and additional copies at cost.

Remittances should be made payable to American Journal of Botany. Ten cents must be added to all checks not drawn on New York City banks.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

Correspondence concerning editorial matters should be addressed to Prof. C. E. Allen, University of Wisconsin, Madison, Wisconsin.

Business correspondence, including notice of change of address, and directions concerning reprints, should be addressed to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y., or 41 North Queen Street, Lancaster, Pa.

AMERICAN
JOURNAL OF BOTANY

VOL. VIII OCTOBER, 1921 No. 8

THE VASCULAR ANATOMY OF HEMITRIMEROUS SEEDLINGS OF PHASEOLUS VULGARIS

J. ARTHUR HARRIS, EDMUND W. SINNOTT, JOHN Y. PENNYPACKER, AND G. B. DURHAM

(Received for publication January 17, 1921)

INTRODUCTORY

In an earlier paper[1] we discussed the gross vascular anatomy of dimerous and trimerous seedlings of the garden bean. By *dimerous* seedlings we understand those of the normal type, characterized by two cotyledons and two primordial leaves, both sensibly opposite in insertion. By *trimerous* we mean those which have a whorl of three cotyledons and three primordial leaves. The cotyledons may be, and frequently are, more or less irregular in insertion. The primordial leaves are, in the seedlings considered, inserted in a regular whorl.

In addition to these two types of seedlings, those which are in a sense intermediate in superficial structure between the two types hitherto studied may occur. These are seedlings with a whorl of three cotyledons but with a normal pair of primordial leaves instead of three as in the case of trimerous seedlings. These we have called *hemitrimerous.* They are extremely rare in occurrence, but during the four years during which these studies have been under way a number sufficiently large to justify a brief discussion of their gross vascular anatomy has been secured.

Our purpose in this paper is to compare the anatomy of these hemitrimerous seedlings with the trimerous seedlings (in common with which they have three cotyledons) on the one hand and with dimerous seedlings (in common with which they have two primordial leaves) on the other.

For convenience of reference the three types will in some cases be designated by the number of cotyledons and primordial leaves: 2–2 = dimerous, 3–3 = trimerous, and 3–2 = hemitrimerous.

MATERIALS

The hemitrimerous plants and the trimerous and dimerous seedlings with which they are compared were largely secured in the series of germina-

---

[1] Harris, J. Arthur, Sinnott, E. W., Pennypacker, J. Y., and Durham, G. B. The vascular anatomy of dimerous and trimerous seedlings of *Phaseolus vulgaris.* Amer. Jour. Bot. **8:** 63–102. 1921.

[The **Journal** for July (**8:** 323–374) was issued August 31, 1921]

tions which furnished the materials for our earlier discussion of dimerous and trimerous seedlings. The dimerous and hemitrimerous seedlings were derived from the same parent plants in lines 75, 93, and 98. In lines 29, 139, and 143 the germinations were made from mass seed instead of from the seed of individual parent plants. All of the seed, however, was grown in the same experimental field in 1917.

Since it has been shown in an earlier paper[2] that there is practically no correlation between the anatomical characters of the trimerous and dimerous seedlings from the same parent plant, we are fully justified in using random samples of hemitrimerous, trimerous, and dimerous seedlings for a comparison of their vascular characters.

A detailed account of the vascular topography of the dimerous and trimerous seedling is presented in a previous paper by the writers, but may be summarized very briefly here. Each primary polar bundle of the root bifurcates in the base of the hypocotyl to form a "primary double bundle," which gives rise to two distinct and well separated strands in the central region of the hypocotyl. In addition to these, there are usually present in the hypocotyl a number of "intercalary" bundles, arising either *de novo* or by splitting of some of the primary strands. At the cotyledonary node a rather complex vascular anastomosis takes place, from which the cotyledonary strands depart and out of which the vascular system of the epicotyl is organized.

PRESENTATION AND ANALYSIS OF STATISTICAL DATA

Base of Hypocotyl

The frequency distribution of the various types of vascular organization at the base of the hypocotyl is shown for all the available data in table 1. In this table the number of primary double bundles appears in parentheses, while the number of intercalary bundles follows the + sign.

Because of the relatively small numbers of hemitrimerous seedlings which can be obtained and because of the irregularity of the frequency distributions for bundle number, it has not seemed desirable in this paper to consider the frequency distributions of the numbers of bundles of the several types. Neither has it seemed desirable, on the basis of the relatively small series of hemitrimerous seedlings which can be obtained, to consider the relative variabilities of bundle number in the different regions of the three types of seedlings as we did in our discussion of variation in the dimerous and trimerous types. We have, therefore, limited ourselves to a comparison of mean bundle number, leaving the question of variability until larger series of countings can be obtained.

---

[2] Harris, J. Arthur, Sinnott, E. W., Pennypacker, J. Y., and Durham, G. B. Correlations between anatomical characters in the seedling of *Phaseolus vulgaris*. Amer. Jour. Bot. **8**: 339–365. 1921.

TABLE 1.

Base of Hypocotyl	Line 29			Line 75			Line 98			Line 139			Line 143		
	3–3	3–2	2–2	3–3	3–2	2–2	3–3	3–2	2–2	3–3	3–2	2–2	3–3	3–2	2–2
(4) + 0. .	—	1	83	—	—	101	—	—	117	—	2	270	2	3	291
(4) + 1. .	—	4	11	—	2	39	—	1	55	1	5	26	3	6	102
(4) + 2. .	1	2	1	—	1	14	—	1	32	—	—	6	—	—	5
(4) + 3. .	1	—	—	—	—	4	—	—	2	—	—	—	—	—	—
(4) + 4. .	1	—	—	—	—	2	—	—	—	—	—	—	—	—	—
(4) + 5. .	—	—	—	—	—	2	1	—	—	—	—	—	—	—	—
(4) + 6. .	—	—	—	1	—	1	—	—	—	—	—	—	—	—	—
(4) + 7. .	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—
(5) + 0. .	7	11	1	1	4	13	4	3	7	4	2	1	15	26	13
(5) + 1. .	6	7	3	8	7	9	6	5	8	4	11	2	31	20	7
(5) + 2. .	—	—	—	2	—	3	1	2	1	—	—	—	—	—	—
(5) + 3. .	—	—	—	—	—	1	—	2	1	—	—	—	—	—	—
(6) + 0. .	39	16	—	107	32	7	160	24	1	92	20	—	134	52	—
(6) + 1. .	—	1	—	12	6	2	10	2	1	5	2	—	25	6	1
(6) + 2. .	—	—	—	2	2	—	—	1	—	—	—	—	—	—	—
(6) + 4. .	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—
(7) + 0. .	1	—	—	7	2	1	1	1	—	—	—	—	5	1	1
(7) + 1. .	—	—	—	—	—	—	—	1	—	—	—	—	4	—	—
(7) + 2. .	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—
(8) + 0. .	—	—	—	1	—	—	—	—	—	—	—	—	1	—	—
(8) + 1. .	—	—	—	—	—	—	—	—	1	—	—	—	1	—	—
	56	43	99	142	57	199	183	43	226	106	42	305	221	114	420

Table 2 shows the average number of primary double bundles, intercalary bundles, and total bundles in the three types of seedlings, and gives the differences and probable errors of differences in the means upon which we must depend for conclusions.

The entries in the first section of this table show that the average number of primary double bundles is relatively lower in the hemitrimerous than in the trimerous seedlings. It is also relatively higher than the number in the dimerous seedlings. The differences, while small, may reasonably be considered significant in comparison with their probable errors. The differences between the hemitrimerous and the dimerous class are much larger than those between the hemitrimerous and the trimerous.

Turning to the statistical constants for intercalary bundles set forth in the second section of table 2, we note that in four of the five cases the hemitrimerous seedlings have a larger number of intercalary bundles than the trimerous seedlings. These differences are small, but may be significant. In the one case in which the hemitrimerous seedlings have a smaller number of intercalary bundles than the trimerous plantlets the difference is only -0.01 ± 0.04. In two of the cases the hemitrimerous show a larger number of intercalary bundles than the dimerous seedlings, but in three lines the reverse is true. The differences are in general not so large in comparison with their probable errors as in the case of the comparison for number of primary double bundles.

TABLE 2. *Mean number of bundles at base of hypocotyl*

	f	Primary Double Bundles	Intercalary Bundles	Total Bundles
Line 29				
3–3	56	5.68 ± .05	.27 ± .07	5.95 ± .04
3–2	43	5.21 ± .08	.53 ± .12	5.74 ± .10
2–2	99	4.04 ± .01	.16 ± .03	4.20 ± .03
(3–2)–(3–3)		− 0.47 ± .09	+ .26 ± .14	− 0.21 ± .11
(3–2)–(2–2)		+ 1.17 ± .08	+ .37 ± .12	+ 1.54 ± .10
Line 75				
3–3	142	5.98 ± .02	.25 ± .04	6.23 ± .03
3–2	57	5.74 ± .05	.44 ± .07	6.18 ± .07
2–2	199	4.24 ± .03	.62 ± .05	4.85 ± .05
(3–2)–(3–3)		− 0.24 ± .05	+ .19 ± .08	− 0.05 ± .08
(3–2)–(2–2)		+ 1.50 ± .06	− .18 ± .09	+ 1.33 ± .09
Line 98				
3–3	183	5.93 ± .01	.13 ± .02	6.06 ± .02
3–2	43	5.67 ± .07	.53 ± .09	6.21 ± .08
2–2	226	4.11 ± .02	.62 ± .03	4.73 ± .04
(3–2)–(3–3)		− 0.26 ± .07	+ .40 ± .09	+ 0.15 ± .08
(3–2)–(2–2)		+ 1.56 ± .07	− .09 ± .09	+ 1.48 ± .09
Line 139				
3–3	106	5.91 ± .02	.09 ± .02	6.00 ± .02
3–2	42	5.36 ± .08	.43 ± .05	5.79 ± .06
2–2	305	4.01 ± .00	.13 ± .02	4.14 ± .02
(3–2)–(3–3)		− 0.55 ± .08	+ .34 ± .05	− 0.21 ± .06
(3–2)–(2–2)		+ 1.35 ± .08	+ .30 ± .05	+ 1.65 ± .06
Line 143				
3–3	221	5.81 ± .03	.29 ± .02	6.10 ± .03
3–2	114	5.45 ± .04	.28 ± .03	5.73 ± .04
2–2	420	4.06 ± .01	.29 ± .02	4.35 ± .02
(3–2)–(3–3)		− 0.36 ± .05	− .01 ± .04	− 0.37 ± .05
(3–2)–(2–2)		+ 1.39 ± .04	− .01 ± .04	+ 1.38 ± .04

We cannot, therefore, assert on the basis of the data now in hand whether dimerous, hemitrimerous, and trimerous seedlings differ in the number of intercalary bundles at the base of the hypocotyl. In so far as it goes the evidence *suggests* that the hemitrimerous seedlings have a larger number of intercalary bundles than the trimerous but a smaller number than the dimerous plantlets.

The means for total number of bundles (primary double bundles plus intercalary bundles) at the base of the hypocotyl set forth in the third section of table 2 show that in four of the five cases the mean number of bundles is lower in the hemitrimerous than in the trimerous seedlings. The differences are, however, very slight indeed and cannot in general be considered significant in comparison with their probable errors. The differences between the hemitrimerous and dimerous seedlings on the other hand are rather large and in every case are unquestionably significant.

Summarizing these results, we note that the hemitrimerous seedlings are conspicuously differentiated from the dimerous seedlings in the number of primary double bundles and in the total number of bundles. They are less conspicuously differentiated, if at all, in number of intercalary bundles. They are unquestionably differentiated from the trimerous seedlings by their lower number of primary double bundles and possibly by a higher number of intercalary bundles. They cannot be said to differ from the trimerous seedlings in the total number of bundles at the base of the hypocotyl.

Central Region of Hypocotyl

For the number of bundles in the central region of the hypocotyl we have the fundamental frequency distributions given in table 3. Considering the mean number of bundles in table 4, it appears that the number of bundles in the central region of the hypocotyl of hemitrimerous plants is slightly lower than that found in trimerous seedlings in four of the six lines available. The differences are, however, small and would not for the most part be considered significant in comparison with their probable errors. The bundle number of hemitrimerous plants is in every case distinctly higher than that of dimerous plants at this level, and these differences are conspicuous and unquestionably significant. Thus in hypocotyledonary structure the hemitrimerous seedling is very close indeed to the trimerous but perhaps shows a slight deficiency in bundle number.

This result is not surprising in view of the fact that so far as the cotyledonary node and lower portions of the axis are concerned the external form of hemitrimerous and trimerous seedlings is essentially identical.

Central Region of Epicotyl

If a differentiation between the hemitrimerous and trimerous seedlings obtains anywhere, one would expect to find it in the epicotyledonary region,

TABLE 3. *Distribution of number of bundles in central region of hypocotyl*

	8	9	10	11	12	13	14	15	16	17	18	20	24	Total
Line 29														
3-3	—	—	1	6	41	1	3	3	—	—	—	—	1	56
3-2	—	2	6	8	21	2	2	—	1	1	—	—	—	43
2-2	67	21	9	2	—	—	—	—	—	—	—	—	—	99
Line 75														
3-3	1	3	5	36	292	40	29	5	1	4	—	—	—	416
3-2	—	2	3	13	51	16	14	2	2	—	—	—	—	103
2-2	177	131	103	46	31	14	11	1	1	3	1	—	—	519
Line 93														
3-3	—	—	8	32	382	82	38	12	1	—	1	1	—	557
3-2	—	—	4	6	17	8	7	1	—	—	—	—	—	43
2-2	36	93	170	107	96	40	18	1	—	—	2	—	—	563
Line 98														
3-3	—	1	6	12	297	21	8	—	—	—	—	—	—	345
3-2	—	—	3	7	25	3	3	1	1	—	—	—	—	43
2-2	125	126	83	37	11	5	—	—	—	1	—	—	—	388
Line 139														
3-3	—	—	4	8	84	6	3	1	—	—	—	—	—	106
3-2	1	3	4	11	19	3	1	—	—	—	—	—	—	42
2-2	269	23	7	4	1	1	—	—	—	—	—	—	—	305
Line 143														
3-3	2	1	11	14	136	21	25	6	3	1	1	—	—	221
3-2	1	2	17	19	54	4	8	5	4	—	—	—	—	114
2-2	263	83	47	17	4	3	1	2	—	—	—	—	—	420

since the sole superficial difference between the two types of seedlings is found at the primordial node. The frequency distributions in table 5 show that the nodal number of bundles is in general lower in the hemitrimerous than in the trimerous seedlings. It also indicates that they are higher in the hemitrimerous than in the dimerous seedlings. The averages and their probable errors in the second section of table 4 show that in each of

TABLE 4. *Mean number of bundles in central regions of internodes*

Line	*f*	Central Region of Hypocotyl	Central Region of Epicotyl	Line	*f*	Central Region of Hypocotyl	Central Region of Epicotyl
Line 29				Line 98			
3-3	56	12.36±.16	14.75±.18	3-3	345	12.03±.02	14.89±.04
3-2	43	11.74±.16	12.93±.17	3-2	43	12.07±.12	13.72±.13
2-2	99	8.45±.05	12.05±.02	2-2	388	9.24±.04	12.12±.01
(3-2)-(3-3)		− .62±.23	− 1.82±.25	(3-2)-(3-3)		+ .04±.12	− 1.17±.14
(3-2)-(2-2)		+ 3.29±.17	+ .88±.17	(3-2)-(2-2)		+ 2.83±.13	+ 1.60±.13
Line 75				Line 139			
3-3	416	12.19±.03	15.47±.04	3-3	106	11.99±.05	15.24±.08
3-2	103	12.32±.08	13.85±.10	3-2	42	11.36±.12	13.93±.18
2-2	519	9.52±.05	12.26±.02	2-2	305	8.19±.02	12.10±.01
(3-2)-(3-3)		+ .13±.09	− 1.62±.11	(3-2)-(3-3)		− .63±.13	− 1.31±.20
(3-2)-(2-2)		+ 2.80±.09	+ 1.59±.10	(3-2)-(2-2)		+ 3.17±.12	+ 1.83±.18
Line 93				Line 143			
3-3	557	12.29±.03	15.65±.04	3-3	221	12.29±.06	16.10±.08
3-2	43	12.26±.13	14.84±.18	3-2	114	11.89±.10	13.68±.09
2-2	563	10.62±.04	12.19±.02	2-2	420	8.66±.04	12.36±.02
(3-2)-(3-3)		− .03±.13	− .81±.18	(3-2)-(3-3)		− .40±.12	− 2.42±.12
(3-2)-(2-2)		+ 1.64±.14	+ 2.65±.18	(3-2)-(2-2)		+ 3.23±.11	+ 1.32±.09

TABLE 5. *Distribution of number of bundles in central region of epicotyl*

	10	11	12	13	14	15	16	17	18	19	20	21	22	Total
Line 29														
3-3	1	—	3	11	13	15	4	2	4	1	1	1	—	56
3-2	2	3	19	4	6	6	2	1	—	—	—	—	—	43
2-2	—	—	97	—	1	1	—	—	—	—	—	—	—	99
Line 75														
3-3	—	—	3	16	63	164	93	41	27	4	4	1	—	416
3-2	—	3	16	28	27	14	9	4	1	—	1	—	—	103
2-2	1	4	422	58	21	10	3	—	—	—	—	—	—	519
Line 93														
3-3	—	—	5	18	47	236	129	56	51	10	4	—	1	557
3-2	—	—	5	4	9	13	5	3	2	2	—	—	—	43
2-2	1	6	483	42	20	10	1	—	—	—	—	—	—	563
Line 98														
3-3	—	—	8	24	69	176	49	9	7	1	1	1	—	345
3-2	—	1	6	12	13	8	2	1	—	—	—	—	—	43
2-2	—	—	352	27	7	1	1	—	—	—	—	—	—	388
Line 139														
3-3	—	—	—	8	21	38	24	9	4	2	—	—	—	106
3-2	—	2	6	12	8	6	5	1	2	—	—	—	—	42
2-2	—	—	278	23	4	—	—	—	—	—	—	—	—	305
Line 143														
3-3	—	—	5	9	19	54	49	37	31	9	6	2	—	221
3-2	—	—	31	19	37	17	6	2	—	2	—	—	—	114
2-2	—	—	318	66	27	5	4	—	—	—	—	—	—	420

the six lines the average number of bundles in the epicotyl is significantly lower in the hemitrimerous than in the trimerous seedlings, and (probably) significantly higher in the hemitrimerous than in the dimerous seedlings.

In epicotyledonary structure the hemitrimerous seedlings occupy as a matter of fact almost exactly an intermediate position between the dimerous and the trimerous types.

SUMMARY

The purpose of this paper is a comparison of the gross vascular anatomy of hemitrimerous seedlings of *Phaseolus vulgaris* with those which are trimerous and those which are dimerous. By dimerous seedlings we understand those with *two* cotyledons and *two* primordial leaves, by trimerous seedlings those with *three* cotyledons and *three* primordial leaves, and by hemitrimerous seedlings those with *three* cotyledons and *two* primordial leaves. The hemitrimerous is, therefore, intermediate in external form between the dimerous and the trimerous seedling. In the internal structure of the axis at the transition zone, which here occurs at the base of the hypocotyl, the hemitrimerous seedling is clearly differentiated from the trimerous type by a slightly smaller average number of primary double bundles, and possibly by a slightly larger number of intercalary bundles. The total number of bundles in the basal region of the axis of hemitrimerous seedlings is not sensibly different in hemitrimerous and trimerous plantlets. The hemitrimerous are conspicuously differentiated from the dimerous seedlings by a larger number of primary double bundles and a larger total number of bundles. On the basis of the data available they cannot be asserted to differ significantly from the dimerous plants in the number of intercalary bundles.

In the central region of the hypocotyl, the vascular anatomy of the hemitrimerous seedling conspicuously exceeds that of the dimerous in bundle number but agrees very closely indeed with that of the trimerous plantlet, although it may have a slightly lower average number of bundles.

In the central region of the epicotyl the mean number of bundles in the hemitrimerous seedling is, roughly speaking, intermediate between that of the trimerous and that of the dimerous types.

Recapitulating, it appears that in internal structure the hypocotyl of the hemitrimerous seedling is practically identical with that of the trimerous seedling with which it has in common a whorl of three cotyledons. The epicotyledonary internode in the hemitrimerous seedling, limited by a trimerous cotyledonary and a dimerous primordial node, is intermediate in anatomy between the trimerous type with three cotyledons and three primordial leaves and the dimerous type with two cotyledons and two primordial leaves.

THE EFFECT UPON PERMEABILITY OF POLYVALENT CATIONS IN COMBINATION WITH POLYVALENT ANIONS

Oran L. Raber

(Received for publication January 20, 1921)

In most of the studies carried on with a view of ascertaining how bivalent ions (such as calcium and magnesium), and trivalent ones (such as aluminum) affect permeability, the anions employed have been monovalent ones (chloride, nitrate, etc.). In studies in which Laminaria has been used, it has been found by Osterhout (1, 2) that wherever bivalent and trivalent cations were employed, the first effect of the salt has been to cause an increase in the resistance of the tissue (followed by a decrease), while if monovalent cations are used (with exception of acids and sodium taurocholate) the first effect is a decrease in resistance.

Since the anions, as shown in previous papers (3, 4) seem to play an important part in determining the permeability of the tissue, it seems of interest to investigate the effect of a salt composed of a polyvalent cation and a polyvalent anion.

The number of such salts which are sufficiently soluble for the present experiments is exceedingly limited, because of the pronounced relation between valency and solubility. Magnesium citrate, magnesium sulphate, and aluminum citrate were finally selected for study since they possess more than most others the requisite characteristics for this work, in respect to acidity, solubility, osmotic pressure, etc.

The solution of magnesium sulphate used was about 1.09 M and had the same conductivity as sea water. Its pH value is about 8 and it is hence only very slightly alkaline. Figure 1 (curve *A*) shows the average result of the six experiments performed. The probable error of the mean (as based on Peter's formula) is under 5 percent of the mean. The rise in resistance at the start is seen to be very small and temporary, and at the end of five minutes the resistance has dropped to 84 percent of the original value in sea water.

With magnesium citrate the difficulty of solubility was encountered. Since it was impossible to get a solution of the same conductivity as sea water, the practice of diluting with chloride was resorted to. When a solution composed of one sixth magnesium citrate 0.16 M and five sixths magnesium chloride 0.28 M is used, the results are as shown in figure 1, *B* (three experiments; probable error of the mean under 5 percent of the mean).

If this experiment is repeated, using magnesium chloride of the same electrical conductivity as the previous mixture, *viz.*, about 0.24 M, the

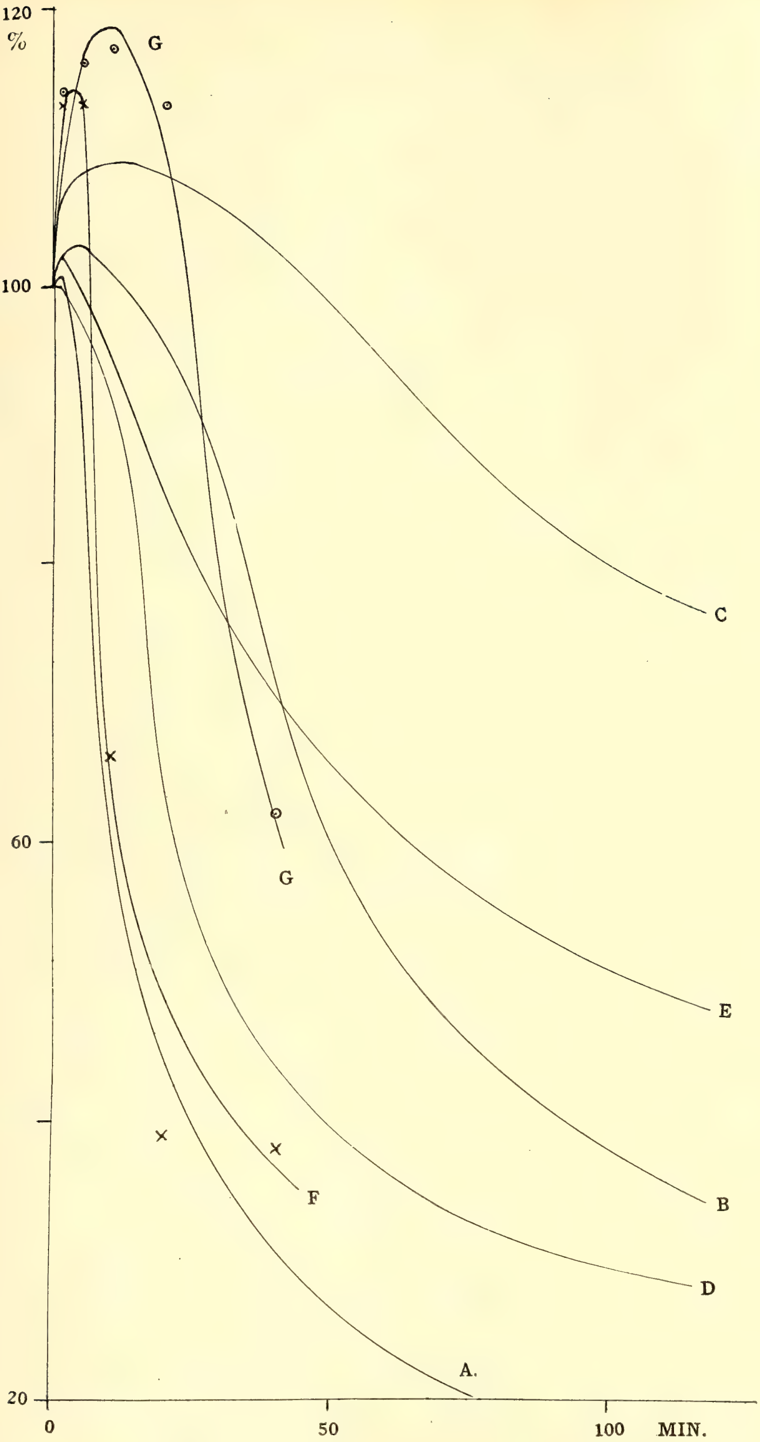


FIG. 1. Curves showing the resistance of Laminaria in solutions of various salts: *A*, in magnesium sulphate; *B*, in one sixth magnesium citrate and five sixths magnesium chloride; *C*, in magnesium chloride of the same conductivity as that represented by curve *B*; *D*, in one third magnesium citrate and two thirds magnesium chloride; *E*, in magnesium chloride of the same conductivity as that represented by curve *D*; *F*, in one third aluminum citrate and two thirds aluminum chloride; *G*, in aluminum chloride of the same conductivity as that represented by curve *F*. Ordinates represent resistance (expressed as percentage of the resistance in sea water, which is taken as 100 percent). Abscissae represent time in minutes.

resistance changes as shown in figure 1, *C* (three experiments; probable error less than 3 percent). It is seen that without the citrate the rise in resistance is much higher than with the citrate. In other words, the citrate has a decided tendency to keep the resistance from rising at the start.

If the proportion of citrate in the mixture is now increased from one sixth to one third, results are obtained as shown in figure 1, *D* (three experiments; probable error less than 3 percent).

A 0.20 M solution of magnesium chloride has about the same conductivity as the last mentioned solution, and if tissue is placed in it the results are as shown in figure 1, *E* (three experiments; probable error less than 5 percent). Here it is seen that the resistance rises slightly in the 0.20 M solution of magnesium chloride, but that if enough magnesium citrate is added the resistance does not increase, but decreases from the beginning. This is of interest since it shows the importance of the anion in studies on permeability.

Figure 1, *F* shows the changes in resistance in a mixture composed of two thirds aluminum chloride 0.40 M and one third aluminum citrate 1.09 M. This mixture has a pH of about 3 and the conductivity of a solution of 63 percent sea water plus 37 percent distilled water. The curve shows the average of two experiments (in which the probable error of the mean is under 3 percent of the mean).

Figure 1, *G* shows the resistance in a solution of pure aluminum chloride of the same conductivity as that represented by curve *F*. The number of experiments and the limit of probable error are also the same. The pH of this solution is about 4.

Since it is known that acid produces an initial rise in resistance, it might be thought that the acidity of the solutions is the chief factor in the effects produced. If this were true, we should expect that the greater the concentration of the citrate the greater would be the initial rise in resistance since the citrate is the more acid of the two salts, but this is just the reverse of what is found to be the case.

Again it may be suggested that in mixtures of magnesium chloride and citrate and of aluminum chloride and citrate synergetic effects are present which cause especially rapid decrease in resistance. Since pronounced synergy has been found between the chloride and citrate of sodium (5), this is quite possible. The possibility of synergetic action can not be denied, but even if it is present it is evident that the anion has a powerful influence. Moreover, the experiments with magnesium sulphate (where there is no possibility of synergetic effects) show that bivalent cations may be prevented from causing an appreciable increase in resistance if they are used with bivalent anions.

Summary

Bivalent and trivalent cations in combination with monovalent anions produce an increase in the electrical resistance of Laminaria, but when

combined with bivalent or trivalent anions the increase is less and may be entirely lacking.

Laboratory of Plant Physiology,
Harvard University.

BIBLIOGRAPHY

1. **Osterhout, W. J. V.** The effect of some trivalent and tetravalent kations on permeability. Bot. Gaz. **59**: 464–473. 1915.
2. ——. On the decrease of permeability due to certain bivalent kations. Bot. Gaz. **59**: 317–330. 1915.
3. **Raber, Oran L.** A quantitative study of the effect of anions on the permeability of plant cells. Jour. Gen. Physiol. **2**: 535–539. 1920.
4. ——. The antagonistic action of anions. Ibid. **2**: 541–544. 1920.
5. ——. The synergetic action of electrolytes. Proc. Nat. Acad. Sci. **3**: 682–685. 1917.

THE FLORAL ANATOMY OF THE URTICALES

Albert Reiff Bechtel

(Received for publication February 9, 1921)

In the search for natural relationships in the seed plants, the morphologist and the anatomist have contributed much to the subject from work on the reproductive mechanism and on the structure of the vegetative organs. During recent years the breeder and the geneticist have presented theories based on experimental evidence which the taxonomist can not ignore. The subject of flower anatomy, however, has been limited in workers and in material studied. This subject should have contributions of value to offer for principles that are to be the guides in determining relationships within the angiosperms, as well as casting more light on the floral characters of the ancestors of the angiosperms.

There is as yet no definite knowledge of the ancestry of the various divisions of the angiosperms. Here hypotheses must be made from the study of external form upon which the classification has been mainly based, and these hypotheses must be strengthened or weakened by evidence from internal structure.

Different views have been held as to what the primitive flower was like. De Candolle (6) looked upon the primitive flower as hermaphroditic with all parts free. Engler (9) considered the simple, naked, unisexual flower as the primitive type. Bower proposed a bisexual flower with many sporophylls surrounded by one whorl of floral envelopes. The types of flowers known to us have been derived from ancestors, undoubtedly, like unto one or more of those above described, by the processes of amplification and reduction, which processes students of natural history accept as the great factors in molding our present forms of life.

Two cautions are suggested by Engler and Gilg (10) for students of phylogenetic relationships to heed: first, the consideration of a simple structure as primitive when it is reduced; second, the placing of reduced forms because of their reduction, too high in rank. There is a guide in this matter of interpreting reduced forms in Bower's (4) definitions:

> Where the development of the natural organism, either in whole or in part, in external form or internal structure, falls short of that of the ancestry, that condition would be described as reduced.

Examples from among the earlier workers of the contribution of floral anatomy to the knowledge of the morphology and relationships of angiosperms are the following. In 1862, Darwin (5) declared the discovery of the nature of the orchid flower by studying transverse and longitudinal sections. His conclusions drawn at that time constitute our present interpretation of the column and labellum. Gérard (11) in 1879 presented the anatomical features of numerous genera of the Orchidaceae. Upon Darwin's discovery it was at once suggested that the Orchidaceae are probably in the same line of descent with the Amaryllidaceae. In all characters the genera agree anatomically except in the androecium, which varies in position and in the number of the stamens and staminodes. Van Tieghem (20) in 1868 published the results of his extensive anatomical work on the flower, particularly on the structure of the pistil. This study treats of the various positions of the ovary with reference to other parts of the flower.

A search into flower anatomy should aid in revealing the following points: whether the same organs in different plants have taken on different functions; whether different organs perform the same function, or whether different forms of the same organ perform the same function (10); what is the condition of the vascular supply to aborted organs and to suppressed organs; whether any amplification in the floral organs has occurred; and what is the relative position of the floral organs, normal and abnormal.

The order Urticales, a study of which forms the subject of this paper, has been nearly universally accepted as a natural primitive order, but has been placed otherwise, bodily or in part, by the following students of taxonomy: Weddell (21), Lindley (13), Bessey (3), and Hallier (12). Although these students have not looked upon this order as among the very primitive angiosperms, they do agree that it belongs among the lower archichlamydeous forms. This study of the group is based upon the anatomy of the flowers and has as its object the possible determination of the phylogenetic position of this group so far as evidence may be offered by this field of research in conjunction with other parallel evidence.

Two treatments of the Urticales are in use to-day: that of Bentham and Hooker (2), which places all species in one family, the Urticaceae; and that of Engler (9), which divides the order into three families: Ulmaceae, Moraceae, and Urticaceae. For convenience in this paper the latter treatment is used.

The following characters possessed by this order of plants have caused them to be looked upon as primitive: flowers usually unisexual; floral envelopes composed of one whorl, which is generally spoken of as the calyx, and which is inconspicuous, bracteal, with parts similar and distinct or gamophyllous; stamens isostemonous; ovary superior, one-celled and one-ovuled, the ovule commonly orthotropous. In spite of these fairly primitive characters these plants have nevertheless been looked upon as somewhat reduced forms, the very features considered primitive being viewed as simple by reduction. The evidence from which such a conclusion is drawn is: apetaly and one whorl of stamens (Bessey, 3); a pistil with a unilocular ovary but with two styles, an indication of two fused carpels. Syncarpy is not considered primitive nor is the solitary ovule (3, 9); these are, on the contrary, the result of reduction. In the present paper are shown cases in which the presence of one style in this order has prompted earlier students of taxonomy to infer the presence of but one carpel; internal anatomical study, however, reveals the unquestioned presence of two carpels.

MATERIAL

The flowers in this study were killed in chrom-acetic acid, embedded in paraffin in the usual way, and sectioned serially, the sections being eight and ten microns thick. Because the flowers are very small and ephemeral the vascular supply is very delicate and not readily differentiated. Experimentation with various stains proved the safranin and light green combination to be the most practical.

ULMACEAE

Ulmus. The species of this genus have flowers arranged in the inflorescence in a graded series from a short, slender, raceme-like cluster or a loose panicle-fascicle, with slender, jointed pedicels as in *Ulmus americana* and *U. racemosa*, to a very much reduced fascicle with pedicels practically eliminated as in *U. fulva*, *U. scabra*, and *U. campestris*. Taxonomists state, usually, that the flowers are simple with bell-shaped perianth, which is 9- to 4-lobed, imbricated, stamens opposite to, and of the same number as, the lobes, hypogynous, inserted at the base of the perianth; pistil of two carpels, each with a style, one loculus with a pendulous anatropous ovule.

The one-whorled perianth of Ulmus can be called neither sepaloid nor petaloid; it is succulent to membranous-scarious with little or no chlorophyll, possessing stomata equally distributed over its outer surface.

Ulmus americana L. has a flower (Pl. XV, fig. 1) which possesses a perianth of 8 lobes, three lateral right and three left, one posterior, and one anterior. Each lobe is accompanied by a stamen. Figure 1 shows the irregular character of the flower. This is a feature usually ignored. Lindley (13) mentions this condition; Britton, in his manual of the northeastern states and Canada describes it as "calyx oblique." This zygomorphy is evident in all the elms studied. Flowers sectioned in transverse planes from the pedicel to the distal end show that the vascular supply to the posterior organs usually passes off from the stele before that of the anterior organs. Such series of sections followed through numerous flowers of *U. americana* reveal the following facts.

The pedicel in all species of Ulmus has an ectophloic siphonostele in the form of a more or less continuous cylinder (Pl. XV, fig. 5). The first break in the stele is on the posterior side where a wide trace passes off leaving a gap in the cylinder. As nearly simultaneously with the break as can be appreciated, there separates from the inner face of this trace a portion composed of one or two vessels (fig. 6, *m*, *n*). The outer bundle is destined to pass to the posterior perianth lobe, and the inner to the accompanying stamen. Fifty microns above this section (fig. 6), the outer strand divides radially into three strands (fig. 7, *m*), and fifty microns above the latter section these strands are well away from the stele and a second trace passes off lateral to the first (fig. 8, m^1) and behaves exactly like the posterior except for the subsequent branching. Twenty microns above the last section a trace, in appearance like the others, passes out of the stele opposite the second trace (Pl. XV, fig. 9, m^2); but twenty microns above this section, as represented in figure 9, the inner portion of this trace appears as a very faintly lignified vessel (figs. 10, 11, n^2), similar in origin and position to those supplying stamens with other lobes. This weak strand aborts seventy microns above its origin. It is clearly the supply to a suppressed stamen. The remaining lateral traces pass off right and left (Pl. XV, figs. 11–13) in close succession. The supplies to the anterior lobe and to the two anterior-lateral lobes appear to pass off simultaneously (Pl. XV, figs. 14–15, m^5, m^6, m^7), and all traces separate into staminal and perianth traces coincident with their departure from the stele. This fact is revealed also by longitudinal sections (Pl. XV, fig. 3, *b*)

When the strands to the perianth and to the stamens are definitely differentiated, such a distinct regional differentiation of tissue arises that the stamen supply is demarked from the adjacent tissues, forming what may be designated as a staminal cylinder (Pl. XVI, figs. 16, 17, *c*). This cylinder persists until the stamens become free from the perianth. At this level the branching of the perianth strands is usually complete; the posterior and anterior separate into two or three strands; the lateral strands rarely separate or branch (Pl. XVI, fig. 18, *m*, m^7).

With a knowledge of the gross morphology of the *U. americana* flower, the above described structure is really what might be expected. However, anatomical study reveals additional abortive bundles present in the anterior half of the flower. These bundles arise immediately within and above the bundles to the stamens and alternate with them. They appear in the same sequence as the bundles to the perianth parts and to the stamens and are evident in transverse sections as soon as the bundles to the stamens are distinctly established in the "cortical" region (Pl. XV, figs. 11–14, d^1, d^2 . . . d^6; fig. 3, d^6; fig. 4, d^2, d^3). These abortive bundles occurring above the bundles to the stamens and below the supply to the carpels show no lignification. They are characterized by cells of small size and by a tissue organization more close than that of the surrounding tissue. The origin of these strands from vascular bundles and the resemblance of the component cells to those of young or weakly developed bundles render their bundle nature undoubted. The centrally located cells of these demarked regions are very small and often exhibit the appearance of being crushed. They thus suggest the appearance of protoxylem as often seen in mature tissue. These abortive bundles extend upward approximately 150 microns. The question arises, are these the vestigial parts of suppressed stamens? There seems to be no alternative conclusion. The same phenomenon is found also with the strands leading to the carpels higher up in the floral axis (Pl. XVI, fig. 16, *e*; Pl. XV, fig. 4, *e*) as discussed below.

Whenever the flower of *U. americana* has an equal number of perianth lobes and stamens, the number is usually 8 (Pl. XVI, fig. 22); occasionally 7, and rarely 9 parts occur in each whorl. In any case the suppression of an organ may occur. Figure 23 shows a flower with 7 perianth lobes and 6 stamens, and figure 24 shows one with 9 lobes and 9 stamens. The lowest number of lobes found in *U. americana* was 7 and the lowest number of stamens was 5.

Small lobes of the perianth frequently exist which are unobserved by means of the hand lens. These occur between and within two main lobes (Pl. XV, fig. 2, *a*). Anatomically, the vascular supply to this small lobe is a branch from a strand to a main lobe. However, there is a possibility that the strand to a main lobe is a union of two or three strands which originally passed to alternating parts of the perianth, and the branch to the small lobe may be the result of a separation of an aggregate strand rather than a case of true branching. Transverse sections frequently reveal indefinite organization of a perianth strand at its origin which may be due to the passing out together of several strands from the floral axis leaving a single gap.

An organized cylinder of vessels continues above the passing off of the traces to the perianth and to the stamens; this first suffers diminution, and then breaks into four strands. This latter change occurs before the stalk of the pistil is isolated from the tissue of the surrounding floral organs (Pl. XV, figs. 14, 15; Pl. XVI, figs. 16, 17). Two of these four strands pass up the posterior and anterior edges of the pistil respectively (Pl. XV, fig. 3, 1, 1^1; Pl. XVI, fig. 17, 1, 1^1) and are the dorsal carpellary bundles. The two remaining bundles, the lateral strands (Pl. XVI, fig. 17, *o*), bear towards the posterior side, approaching each other as they ascend and apparently forming one bundle (Pl. XVI, fig. 18, $o + o$). Serial sections (Pl. XV, fig. 15; Pl. XVI, figs. 16, 17, *o*) show that these strands in present development are apparently a continuation of the axis and not branches of the dorsal carpellary bundles (see discussion, p. 404). Where the pendulous ovule originates this composite strand separates into four strands, one passing into the ovule, one soon vanishing toward the posterior side of the pistil (Pl. XVI, fig. 19, *i*, i^1; Pl. XV, fig. 3, *i*, i^1), and two passing upward. The latter branch again sends bundles into the lateral edges of the two styles (Pl. XVI, figs. 19–21, o^1 and o^2).

This separation of the ovule-bearing strand indicates the probability of the former presence of more than one ovule. The branching at the apex supplying both styles (Pl. XV, fig. 3, o^1, o^2) indicates that these are the supply of an axillary placenta tissue. The existing ovule is in the anterior carpel. The aborted bundle (Pl. XVI, fig. 19, i^1; Pl. XV, fig. 3, i^1) is the remnant of the supply to the ovule that was borne in the posterior carpel and which still is present in some species (Engler, 9; Bentham and Hooker, 2; Baillon, 1).

Ulmus fulva Michx. is described as having perianth lobes and stamens ranging from 9 to 5. However, among the many flowers inspected by the writer, 8 was found to be the highest number and 5 the lowest, with one instance of the latter. The common number is 7 or 6, while in *U. americana* it is 8 or 7. The perianth of *U. fulva* is more prominently lobed than that of *U. americana*, but zygomorphy is as conspicuous. The traces to the floral organs pass off from the stele in close succession, but the traces to the stamens pass off higher up and later than do those to the perianth (Pl. XVII, fig. 1, *b*). *U. fulva* has abortive bundles similar to those of *U. americana*, alternating with the perianth and stamen strands. However, in this species these abortive bundles exist as a distinct whorl and not merely in the anterior part of the flower as in *U. americana*. These are organized tissues in the form of strands (Pl. XVII, fig. 2, *d*; fig. 1, *d*), but they possess no appreciable lignification. They persist to a level where the strands to the stamens are definitely isolated. The flower figured shows the anterior-lateral stamen suppressed, but its abortive trace (Pl. XVII, fig. 2, s^1) can be followed for 30 microns. The vascular supply to the carpels is the same as that of *U. americana*.

Ulmus racemosa Thomas is described as having the perianth lobes and stamens ranging from 8 to 5. All the flowers studied, from one tree only, had perianth lobes 8 to 7 and stamens 8 to 6. The flower tends to be zygomorphic to the same degree as that of *U. americana*. Traces into the floral organs originate and pass off in the same sequence (Pl. XVII, fig. 3, *b*; fig. 4). The presence of abortive bundles alternating with the bundles to the stamens and appearing later and above them was limited in this species to one instance (Pl. XVII, fig. 3, *d*, d^1). Here only two such bundles were found.

Ulmus campestris L. is described as having the perianth lobes and stamens varying from 5 to 3. In flowers from two trees, the perianth lobes were found to range from 6 to 4, and the stamens from 5 to 4; six lobes in the perianth are rare, and the common relationship is 5 to 4, or 4 to 4. The perianth cut away from the flower and studied under a microscope reveals the fact that the lobes are not the single structures that an observer takes them to be when inspecting the flower macroscopically. The hairy margin of the lobes obscures very small lobes on their sides (Pl. XVII, figs. 6, 7, *a*, *b*). In figures 6 and 7, the lobes 2 and 4 are anterior and posterior respectively; lobes 1 and 3 are lateral. Such is the origin and appearance, however, of these lateral veins *a* and *b*, that they should not be looked upon as branches of the midvein, but rather as veins separating from the vein leading to the main lobe. That is, veins *a*, 1, and *b* in figures 6 and 7 are the continuation of the traces, which pass out of the stele contiguously and remain in conjunction for a short distance, separating early. These bundles from their behavior (Pl. XVII, figs. 6, 7, *a*, 1, and *b*) may be considered alternating parts of two perianth whorls, which through reduction have become consolidated except in their distal parts. Stronger evidence for the same conclusion is described for *U. americana* on page 390.

The vascular supply to the flower duplicates that of *U. fulva* (Pl. XVII, figs. 1, 2) and of *U. scabra* (Pl. XVII, figs. 8–10). Commonly one and sometimes two perianth lobes have the stamen suppressed even in the vascular supply.

Ulmus scabra Mill. is described as having the perianth lobes and stamens ranging from 6 to 5; a study of many flowers reveals that 6 perianth lobes and 6 stamens appear in the majority of cases. The vascular supply to the perianth and to the stamens arises separately (Pl. XVII, fig. 8, *b*) as it does in *U. fulva* and in *U. campestris*. The strands to the perianth lobes (Pl. XVII, fig. 9, $m \ldots m^5$) are well out in the "cortical" regions when the supply to the stamens is just passing out of the stele (fig. 2, $n \ldots n^5$).

U. scabra presents a feature not found in any of the other species. Alternating with the traces to the perianth lobes and arising with them are bundles that apparently lag behind (Pl. XVII, figs. 9, 10, x, x^1, x^2; fig. 8, x, x^2; Pl. XVIII, figs. 1, 2, x, x^1, x^2). These are perianth bundles but they always continue inside the strands to the perianth lobes. They do not have the number of lignified cells, nor the size of cells that the strands to the perianth lobes possess. They weaken rapidly and vanish on a level with the origin of the lobes of the perianth. Such bundles were not found in the posterior part of the flower (Pl. XVII, fig. 9). The anterior part of the flower of Ulmus is clearly the conservative part of the flower, since in this part the stamens opposite the perianth lobes are always present. Suppressed stamens occur in the posterior part of the flower, or here the stamen is present and the perianth lobe is suppressed. Also, the abortive bundles described in the above named species usually occur in the anterior part of the flower except in *U. fulva* where they make one complete whorl (Pl. XVII, fig. 2, *d*), but the abortive stamen, s^1, in the same figure, is in the posterior part of the flower. Thus, as described above, *U. scabra* presents an additional feature in the anterior part of the flower, namely, the weak bundles to the perianth lobes. These bundles, x, x^1, x^2, may be vestigial parts of suppressed perianth parts which alternated with the present perianth lobes. The origin, the position, and the appearance of these weak bundles offer no other disposition except that of a vascular supply to corolla parts which have been reduced and consolidated in the gamophyllous perianth.

Alternating with the strands of the perianth, except with the posterior strand, are organized tissue regions as in the other species suggesting bundles, but these show no lignification (Pl. XVII, fig. 9, *d*). The same condition has been fully discussed above in the other species. Again, there seems to be no other alternative here than to look upon these as abortive bundles to a suppressed outer whorl of stamens.

The carpel supply duplicates that of the above named species. Figures 9 and 10 in Plate XVII and figures 1 and 2 in Plate XVIII are from a flower with three carpels developed. This may be looked upon as an abnormality, yet this extra carpel is a character parallel with the seven aborted bundles (Pl. XVII, fig. 9, *d*) alternating with the perianth traces instead of the five or three abortive bundles in flowers which have the usual two carpels. This flower has also three abortive bundles in the perianth (Pl. XVII, figs. 9, 10; Pl. XVIII, figs. 1, 2, *x*, x^1, x^2) which have been considered above as petal traces instead of two or no such traces in the bicarpellate flower.

Celtis occidentalis L. reveals a symmetry of 6 or 5; any other rarely occurs. A unique flower was found with seven perianth lobes and five stamens. This was selected to figure on Plate XVIII. The pedicel as it passes into the flower presents a stele that organizes itself into anterior and posterior sections (Pl. XVIII, figs. 4–6). From both of these sections bulky strands pass out, and each of the latter separates into two strands passing to the perianth lobe and to the stamen respectively (fig. 3, *b*; figs. 6, 7, *m*, *n*). This common origin of the vascular supply of the perianth and stamens and the persistence of this condition for a short distance is a new feature in the Ulmaceae. In addition to this is the unusual origin of the ovule supply. This arises distinctly from the dorsal carpellary strand of the anterior carpel and passes towards the posterior side of the pistil upward into the pendulous ovule (figs. 3, 8–11, *o*). The two lateral strands (figs. 9–12, o^1, o^2) pass up separately and vanish at the base of the styles. The course of these lateral strands is very different from that of the lateral strands in the Ulmus pistil where the lateral strands approach and join to form the ovule supply (Pl. XVI, figs. 17, 18, *o*). Also branches from the ovule-bearing strands in Ulmus continue in the inner lateral edges of the styles, but in Celtis there are no branches from the ovule-bearing strand. In this respect the ovule-bearing strand of Celtis is similar to that of the remaining species of the Urticales studied. The lateral strands of the pistil of Ulmus are, therefore, not homologous with those of the pistil of Celtis although they apparently originate in the same manner. In Ulmus they are the placental supply, but in Celtis they may be regarded as abortive, dorsal, carpellary bundles of suppressed carpels. The placental supply in Celtis arises distinctly from the anterior carpel supply. Evidently reduction in the gynoecium of Ulmus has proceeded to a much greater degree than in Celtis. In Ulmus the placental supply has apparently lost connection with the carpel supply and arises from the axis of the flower (see discussion, p. 404).

The staminate flower of Celtis possesses an abortive pistil, a miniature of the pistil in the hermaphroditic flower, except that the lateral bundles in the pistil (figs. 9–12, o^1, and o^2 above) are not present. The dorsal carpellary bundles of the abortive pistil continue into the two styles. The posterior style is smaller and less succulent than the anterior style. Here is a consistency in abortion, the posterior carpel being the sterile carpel in the hermaphroditic flower and the more greatly reduced carpel in the staminate flower; and the lateral strands of the pistillate flower (figs. 9–12, o^1 and o^2), regarded as dorsal strands of abortive carpels, are suppressed in the abortive pistil of the staminate flower.

Moraceae

In brief, the plants of this family are woody with small flowers usually in dense clusters, unisexual; the perianth 5- to 4-parted, stamens equal in number with, and opposite to, the parts of the perianth; ovary one-celled with single pendulous ovule, styles 2 or 1. The ovule is "basal" (1, 9) in a few species.

Morus alba L. presents in the pedicel of the pistillate flower a stele of four traces (Pl. XIX, figs. 2, 3). From these four traces pass off in a decussate manner the posterior and anterior traces, followed closely by the lateral (fig. 4, p, p^1). These traces supply the four perianth parts, and in each part the bundle separates into three strands (figs. 4, 5, p, p^1). The floral axis above the point of departure of the perianth traces continues as four strands, posterior, anetrior, and two lateral. The anterior and posterior strands are the dorsal bundles of the two carpels (Pl. XIX, figs. 4–9, 1, 1^1) and pass on into the styles. The two lateral strands, as in Ulmus, approach each other as they ascend, unite, and pass upward into the ovule (Pl. XIX, figs. 1, 4–7, o). In *Morus alba* these two strands to the ovule do not receive any evident vascular supply from the anterior carpellary strand as figured by Welsford and Benson (22) for *M. nigra*.

The pedicel of the staminate flower (Pl. XIX, fig. 10) shows many strands in the stele which organize into four strands in the base of the flower. From these, four traces pass off decussately and each soon separates into strands to the perianth parts and to the stamens. The dorsal carpellary supplies persist in the abortive pistil of the staminate flower (Pl. XIX, fig. 10, 1, 1^1).

An interesting difference in the vascular supply to the perianth parts of the pistillate and staminate flowers is that there are three traces to each part in the former and only one in the latter. In the pistillate flower, the perianth persists in the fruit as a fleshy organ and calls for a vigorous vascular supply. The staminate flower functions to the time of pollen production and then falls. As a result, the vascular supply to its perianth has degenerated to a single weak strand in each lobe. This is an illustration of what happens frequently in members of the Urticales. The organ degenerates to the extent that the apparent demand for it decreases.

Maclura pomifera (Raf.) Schneider has its pistillate inflorescence in a dense, succulent head, the individual flowers being sessile. A transverse section of the inflorescence axis below the bases of the flowers shows the many pedicellar steles surrounded by a continuous, extremely delicate, parenchymatous tissue. Each stele is composed of four strands. From these strands there pass off in the base of the flower four traces to the perianth (Pl. XIX, figs. 11, 12, *p*); the remaining four continue into the pistil (figs. 11, 12, 1, 1^1, *o*). The perianth parts become distinct at a level with the ovule (fig. 16), which is not far above the level where the flowers become distinct from each other (fig. 13).

The perianth parts vary much in the amount of vascular supply. In addition to the one main bundle, there frequently exist in the same inflorescence flowers having perianth parts with few to many small, weak bundles (Pl. XIX, figs. 14–17, *n*). The peculiar feature of these bundles is that they cannot be followed to their origin because of the lack of any organization suggesting bundles in the lower part of the perianth. These small strands are either branches of the main bundle of the perianth part, or, as shown in Ulmus, they are a separation of the strands that are now passing off from the floral axis as a common trace. The presence and abundance of these faint bundles vary according to the crowding of the flowers in the dense capitate inflorescence. The two lateral strands to the pistil (Pl. XIX, fig. 12, *o*) approach each other, becoming one strand (figs. 13–16, $o + o$) which passes to the posterior side upward into the pendulous ovule (fig. 18, *o*). The anterior bundle passes up the anterior side of the pistil to the tip of the single filiform style. This is the bundle to the anterior carpel (figs. 12–18, 1). The corresponding bundle passes up three-fourths of the height of the ovary (figs. 12–18, 1^1), on its posterior side. Comparing the vascular supply of the two carpels of Morus with that of Maclura, the conclusion is that the posterior carpel of Maclura is abortive. This abortion of the carpel and the non-actinomorphic condition of the flower make zygomorphy a feature of the Maclura flower.

In the staminate flower four traces from the pedicel separate into strands to the perianth parts and to the stamens. The carpels are suppressed, and there are no signs of any vascular tissue in the central portion of the flower (Pl. XIX, fig. 19).

Cannabis sativa L. The pedicel of the pistillate flower has four stelar strands (Pl. XX, fig. 2). One of these strands (fig. 2, *a*) passes off anteriorly into the bract which completely envelops the flower (figs. 2–4, *br*). The three remaining strands pass up into the pistil of the flower. Two strands (Pl. XX, figs. 2, 3, 1, 1^1) which have nearly the same size pass up the dorsal sides of the two carpels to the tips of the two styles respectively (figs. 2–8, 1, 1^1). The fourth strand of the pedicel, which is opposite the strand passing into the enveloping bract, is twice the size of any of the other strands (Pl. XX, figs. 2–4, *o*). This strand maintains its bulkiness as it passes up posteriorly into the pendulous ovule (figs. 5–7, *o*).

A transverse section of the flower just below the ovule shows six distinctly lignified bundles in the perianth (Pl. XX, fig. 5, *m*). The appearance of the tissues of the perianth suggests more bundles than those having vessels. None of the bundles, even those with lignified cells in their upper portions, can be followed to their origin in the floral axis. The posterior bundle (fig. 4, *m*) can be followed down the farthest, that is, into the cortex of the pedicel or receptacle. It does appear that the perianth bundles are abortive in the lowest part of their courses.

Payer (14) and Zinger (24) describe and figure the cup-like perianth as having slightly developed anterior and posterior lobes. Anatomically, the author found no difference in the lobed regions as compared with the remainder of the perianth except the fact that the most prominent bundle to the perianth is the posterior bundle.

A feature of the pedicellar stele not yet described is the presence of regions suggestive of bundles (Pl. XX, figs. 3, 4, *x*). Such a condition described in the preceding species was looked upon as one demonstrating abortive bundles. Such faint bundles and others not recognizable may pass into the perianth and become lignified in their upper parts only, a condition such that they can be followed. Also in the upper lateral ovary wall there are faint bundles with delicate, lignified cells (Pl. XX, fig. 5, *n*) which cannot be followed to their origin. These must be either branches of the dorsal carpellary bundles or strands continuing from the pedicel. If the latter, they arise similarly to the two dorsal carpellary bundles and therefore suggest abortive carpellary bundles to suppressed carpels.

The stele in the pedicel of the staminate flower is very different. It has many small strands which organize into five strands in the base of the flower. These pass out of the axis and each separates immediately into strands to the perianth and to the stamens. There are no signs of abortive strands to the suppressed carpels in the writer's experience. Likewise in the pistillate flower, the stamens are suppressed and no vestiges of vascular supply are present.

Pritchard (16) concludes from his experiments on the hemp plant that both the male and the female flowers are potentially hermaphroditic and that the unisexual condition is the result not of different zygotic constitution, but of the lack of food supply. At what time in the life of the hemp plant the suggested feeding must be begun in order to establish organs that are suppressed, even in vascular supply, is an interesting problem to a plant anatomist. It may indicate that the unisexual nature of the hemp flower is not well established. Some of its congeners in the order still have bisexual flowers.

Humulus Lupulus L. The pedicel of the pistillate flower duplicates in structure that of Cannabis except that it has fewer vessels in each of its four bundles. Anteriorly, a large trace passes out and branches profusely in a large bract which envelops the flower (Pl. XX, fig. 9, *br*; fig. 10, *a*). The three remaining strands in the pedicel, as in Cannabis, pass into the pistil. Strands (figs. 14–18, 1, 1^1) pass up the dorsal sides of the two carpels into the styles respectively. These are the dorsal carpellary bundles. The remaining strand (figs. 9, 14–16, *o*) passes up posteriorly into the pendulous ovule. The pedicel of Humulus, also, possesses in its upper portion definitely organized tissues suggesting bundles (figs. 10, 11, *x*). One of these suggestive regions does possess a faintly lignified vessel (fig. 10, *x*) which must eliminate all doubt of its being a bundle.

The perianth of Humulus is very similar to that of *Cannabis sativa* Transverse sections through the upper part of the perianth reveal many bundles (Pl. XX, fig. 16, *p*), varying in number from 10 to 14. These bundles cannot be traced to an origin in the pedicellar stele, but they can be followed passing into the cortex. Figures 12, 13, 14, and 15 show the traces to the perianth numbered in the order in which they become distinguishable, *e.g.*, figs. 12, 13, 1^1, 1^2, 1^3. Because of the delicate cell walls, it cannot be definitely said whether there are three, five, or more original traces leading into the perianth. However, it is evident that several traces to the perianth originate at one point in the stele, or that the traces separate after they passed out as one trace (figs. 13–15). Since the perianth strands are difficult to trace owing to their delicacy, we conclude, as for Cannabis, that the basal portions are abortive.

In Humulus, as in Cannabis, the abortive bundles in the pedicel may continue into the perianth and show lignification only in their upper portions. A fact supporting this conclusion is that these abortive bundles appear after the strand to the bract is oriented and before the appearance of the remaining strands that pass into the pistil. In all other species studied in the Urticales, the perianth bundles pass off first or lowest on the floral axis, and this is the position of the abortive bundles of Humulus and Cannabis.

URTICACEAE

This family of the Urticaceae contains perennial or annual herbs with very small, greenish flowers, monoecious, dioecious, or polygamous; perianth parts 5 to 2, distinct, cleft, or tubular; stamens of the same number and opposite to the perianth parts; ovary with one cell, one "orthotropous" ovule; styles usually capitate and sessile.

Urtica gracilis Ait. is figured in Plate XXI. The vascular supply in the minute pedicel of the pistillate flower appears as one strand (fig. 2) which gives off two decussate pairs of bundles which pass to the four perianth parts (figs. 3–12, *m*, *n*). The remaining vascular tissue continues as four strands into the pistil. The posterior strand passes up into the sessile stigma. This strand is the dorsal carpellary bundle of the posterior carpel. The anterior strand passes up three fourths of the height of the ovary and cannot be followed further. The two lateral strands approach each other as they ascend and enter the funiculus of the basal ovule as one strand (figs. 1, 5–11, $o + o$), as was found in Ulmus, Morus, and Maclura. In interpreting the vascular supply of this pistil similarly to that of Ulmus and others previously described, the conclusion is that two carpels are present but that the anterior carpel is partially abortive. However, Urtica has been looked upon as being "unicarpellary" (Baillon, 1; Bessey, 3).

Transverse sections of the Urtica flower indicate zygomorphy: the two lateral perianth parts are alike; but the posterior is larger than the anterior and is the last to become distinct from the shallow perianth tube (figs. 1, 10–13, *m*); the pistil does not stand in the middle of the flower.

Boehmeria cylindrica L. (Sw.) possesses a tubular flower with the vascular supply of the perianth confined to the anterior and posterior sides (Pl. XXII, figs. 1, 3–7, *m*). This fact indicates that the lateral perianth parts have been consolidated with the anterior and posterior parts and that their vascular supplies have completely degenerated. This flower shows the zygomorphic features of the Urticales.

Only two strands pass into the pistil instead of four as in *Urtica gracilis*. These two strands arise from one strand (Pl. XXII, figs. 3, 4, 1^1 and *o*) in the basal portion of the flower. The posterior strand passes up the posterior side of the pistil into the short filiform style and is the dorsal carpellary bundle. The other strand ascends anteriorly for a short distance and then sharply curves towards the posterior; after passing horizontally in this direction for a short distance it abruptly ascends into the "basal" ovule (figs. 1, 5, *o*). The anterior side of the pistil has no vascular supply. Here undoubtedly the anterior carpel is suppressed. Therefore, *Boehmeria cylindrica* has reached that stage in reduction having only two perianth parts, one carpel, and a "basal" ovule. The ovule from the path of its bundle (fig. 1, *o*) indicates that its apparent orthotropous nature has become such by a sinking down to a basal position from a pendulous or lateral position.

In the base of the staminate flower, the strands of the pedicel conjoin (Pl. XXII, figs. 9, 10, 11) and strands then pass off to the perianth and to the stamens (figs. 9, 12, *m*, *s*). The staminate flowers have an abortive pistil which possesses a weak vascular supply (figs. 9, 12, 1, 1^1).

Laportea canadensis (L.) Gaud. has a flower that is decidedly zygomorphic. The floral structures vary from those described above. The anterior perianth part is large, the posterior is very small, and the lateral parts are alike. From a cylindrical stele (Pl. XXII, fig. 15) of the tiny pedicel a strand passes anteriorly into the anterior perianth part (figs. 14, 16, *m*). No strand corresponding to the anterior perianth strand passes off into the small posterior perianth part (figs. 14, 16, m^1). Two strands pass off laterally to the lateral perianth parts. Evidently the decussate arrangement of the perianth supply as exhibited in *Urtica gracilis* is broken in *Laportea canadensis* through the suppression of the posterior perianth trace, although a very small perianth part is still present (figs. 14–19, m^1).

Above the origin of the perianth bundles only two bundles continue, and these in an anterior-posterior plane. The posterior bundle passes up the posterior side of the pistil into the single style (Pl. XXII, figs. 14, 16–21,

1^1). The anterior bundle (figs. 14, 16, 17, *o* + 1) continues for some distance and then separates into two unequal strands, one passing into the anterior side of the pistil and the other, the larger, passing into the ovule (figs. 14, 18–20, *o*, 1). The former strand soon vanishes in the lower third of the ovary wall (fig. 14, 1). This is evidence of an abortive anterior carpel.

The ovule and its vascular supply again offer opportunity for speculation. After the anterior carpel strand separates from the single anterior strand, the main supply passes horizontally in an ascending-posterior direction through a long stocky funiculus into the ovule (figs. 14, 18–20, *f*, *o*). The position of the ovule suggests the reduction of an axillary placenta which bore ovules in a pendulous position. The ovule is past the midway stage between that of a pendulous ovule as in Ulmaceae and Moraceae and that of a basal ovule as in *Boehmeria cylindrica*. In fact, very little reduction in funicular tissue in *Laportea canadensis* is necessary to duplicate in position that of the ovule of Boehmeria (compare Pl. XXII, figs. 1 and 14). The same line of reasoning is suggested upon comparing the ovule supply of *Boehmeria cylindrica* and *Urtica gracilis* (Pl, XXI, fig. 1; Pl. XXII, fig. 1), namely: the bundle in its indirect route to the basal ovule of Boehmeria would require little reduction to duplicate the direct supply to the basal ovule of Urtica.

Discussion

Ulmaceae. The gross floral morphology of the six species and the detailed anatomy underlying it have been presented above. The latter reveals features which warrant the disuse of the descriptive term "simple" for the flowers: five sets of organs or vestiges of organs; variableness in number of parts in a whorl; zygomorphy, which is constant; and the fusion of like and unlike parts. These present a decidedly complex condition.

The flowers of the genus Ulmus have a perianth "cup" upon the edge of which the perianth parts and stamens have been considered perigynously inserted. Baillon (1) considered this to be the condition. Anatomical work reveals that this is not the case for the following reasons. First, the vascular supply to the stamens and to the perianth parts arises separately from the stele of the pedicel; the former passes off from the floral axis considerably above that to the latter in *U. fulva*, *U. campestris*, and *U. scabra* (Pl. XVII, figs. 1, 5, 8, *b*), but approximately closely in *U. americana* and *U. racemosa* (Pl. XV, fig. 3; Pl. XVII, fig. 3, *b*). Second, the tissues embodying the perianth and stamen traces through the perianth "cup" are separable by the distinct difference in cellular structure and by a line of demarcation. These differences in the parenchymatous tissue are collateral and continue into the perianth lobe and stamen respectively. The line of demarcation indicates an adnation of the tissues of the perianth lobe and stamen. Here is good evidence that the "cup" is the fused bases of floral envelopes and stamens. Third, the lobes of the perianth are variable in length; their size is not constant, which is a character not uncommon in hypogynous flowers. The last is among the characters used by Planchon (15) in distributing the 16 species of Ulmus into three divisions.

Celtis has perianth parts distinct to the base or nearly so. The remaining genera of the Ulmaceae, eleven in number, have perianth parts similar to those of Celtis (2). In the Ulmaceae the receptacle is limited, then, to the pedicel of the flower, and in Ulmus, coalescence and adnation have taken place in the perianth parts and stamens. Also, the fact that the single whorl of normal vascular bundles to the perianth and to the stamens, respectively, is accompanied by whorls of abortive bundles which alternate apparently with these, enforces the conclusion that along with the coalescence and adnation, there has been reduction in these two sets of organs. This reduction consists of the loss of an inner whorl in each. Reduction occurs not only in the number of whorls but also in the number of organs within a whorl. No constant number exists in the floral whorls of any of the elms. Greater variation occurs in those species having the greatest number of organs present per whorl; *e.g.*, in *U. americana*, as described on page 390, there are 9 to 7 perianth lobes and 9 to 5 stamens. The cause of the lack of floral symmetry in a species is due to the development of a perianth lobe without its accompanying stamen. This is usually the stamen to one or the other of the posterior-lateral lobes. However, just as often, a stamen develops without an accompanying perianth lobe. The number ranges from 9 in *Ulmus americana* to 4 in *U. campestris*, and is more or less inconstant in all species. On the basis of inflorescence (which shows in Ulmus stages in reduction), the species with more floral parts are more primitive than those with fewer. Although the gynoecium of Ulmaceae is dimerous, from the presence of abortive strands to suppressed carpels it has suffered reduction. Such organized tissue regions suggesting bundles were discovered in *Ulmus americana*. These bundles appear some distance above those to the stamens, on a level from which the strands to the carpels can be followed.

Ulmus possesses spirally arranged parts (Pl. XV, figs. 7–14), though the other genera studied are cyclic. The spiral arrangement is most conspicuous in the species with the greatest numbers of stamens and perianth parts and becomes less conspicuous in those elms in which the floral characters grade into those of the Moraceae which are tetramerous and cylic. The spiral arrangement is an important phyletic character, but by reduction in the number of organs and in the floral axis, it has become nearly obscure.

The alterations in the posterior part of the flower over those of the anterior part by modification in the relation of organs to each other, and by the suppression of organs, form a true zygomorphy. This character is perhaps the result of aggregation (23), and possibly an adaptation to insect visitation. To be sure, very few species in the Urticales are known to be visited by insects, yet zygomorphism may be a character persisting from an earlier time when insect visitation was the common occurrence. There is a possibility that zygomorphism as a specialized character and as a character particularly adapted to insect visitation has been over-emphasized. Evidence has been presented that the angiospermous prototype (Robertson, 17) was entomophilous and that the anemophilous condition has been recently acquired. A character that in many instances accompanies the entomophilous flower is the multiovulate condition. The vascular supply of the placenta of Ulmus, the flowers of which are least reduced of those genera studied, indicates that whereas but one ovule is now borne, a multiovulate condition probably existed formerly.

The characters gamophylly, zygomorphy, bicarpellate, uniovulate ovary, vestigial organs, indicate certain specialization and a high flower type. However, it is only in Ulmus that the gamophyllous character exists. The gamophylly of Ulmus is to be considered an isolated instance of this tendency in the Polypetalae. Finally, from the evidence gathered, the Ulmaceae are primitive forms but with many advanced characters. They should be considered highly reduced and specialized forms among primitive groups.

Moraceae. The flowers of Morus and Maclura are anatomically alike, although the former has a pistil with two styles and the latter a pistil with one style. Anatomy reveals two carpels in each case. This reduction in the gynoecium of Maclura is no doubt a feature accompanying the dense inflorescence. For the same reason the common variation in the size and venation of its perianth parts, as previously described, occurs. The three-veined character of the perianth parts is constant in the Morus pistillate flower but not constant in that of Maclura. This suggests a palmate venation which corresponds to the venation of the foliage leaves. The leaves of Morus, as in many related genera, have three basal veins, and when the foliage leaves are large they have three lobes. According to Sinnott and Bailey (19), palmate venation is the primitive type in the angiosperms, and where it occurs in the floral parts only, as it does inconstantly in Maclura, it is a "persistence of an ancient character which has been lost elsewhere." This sign of primitiveness is conspicuous also in the perianth parts of Ulmus (p. 388). The anterior and posterior perianth parts have three veins as a nearly constant character. The midvein departs first, and soon the lateral veins separate from it.

Humulus and Cannabis form a type distinct from Morus and Maclura. The very delicate gamophyllous perianth in the pistillate flowers (p. 396) has been produced undoubtedly by the large persisting bract which envelops these flowers. Since the vascular supply is evident only in the upper part of the perianth, it is an indication that the perianth is in the process of disappearing. As the venation in the perianth parts of Ulmus and Morus was interpreted by referring to the venation of the foliage of the same, the perianth of Humulus and Cannabis can be so interpreted. The leaves of these two species have palmate venation and both are multi-digitately veined. Thus the many small veins of the perianth of Humulus and Cannabis, the origin of which cannot be determined in the gamophyllous perianth cup, may be considered veins of several digitately veined perianth parts. They attain a weak development due to reduction. The floral envelopes have become reduced and delicate with the development of a large protecting bract. The differences in the ovule supply in these genera will be elaborated upon in the general discussion (p. 404).

Thus, the flowers of the Moraceae as compared with those of the Ulmaceae have been more greatly reduced in floral axis, as described earlier in this paper, in perianth lobes, and in their vascular supply, as seen in Maclura, Humulus, and Cannabis, and in the gynoecium as illustrated in Maclura.

Urticaceae. The study of three species of this family indicates the presence of that reduction which is found in its earlier stages in Moraceae, namely, the suppression of one of two carpels. In Urtica and in Laportea the anterior carpel is represented only by abortive bundles; in Boehmeria there is no trace of a bundle in this carpel. Also the irregularity of the perianth parts is slight in Urtica; it is greater in Laportea to the degree that the posterior perianth part has nearly disappeared, and has no vascular supply. In Boehmeria, an anatomical study of the gamophyllous perianth reveals two perianth parts only. There is no evidence of lobes indicating lateral perianth parts, nor bundle supply to such parts. The "orthotropous" ovule supply, as has been presented on page 398, gives evidence by its peculiar course of a change of position of the ovule from a pendulous or lateral to a basal position. The irregularity of the shape and size of the perianth parts, the number of parts, ranging from four to two, the "basal" ovule supplied by a bundle taking an ascending and then a descending course, indicate a reduction in this family beyond that found in the Moraceae.

Along with the floral reduction in the Urticaceae goes the herbaceous perennial or annual plant habit which character phyletically (7, 18) is in keeping with that of the flower.

General Discussion

The Urticales present an anomalous combination of characters. These on one hand indicate primitiveness and on the other specialization. Many and indefinite organs, non-cyclic condition, preponderance of woody forms, and palmate venation, still evident in the perianth parts if not in foliage leaves, point to primitiveness. Aggregation of flowers, fusion of parts, zygomorphy, and reduction point to specialization. Therefore, they must be considered at least not highly advanced forms, though they possess a number of very advanced features. Almost any group of angiosperms possesses one or more of the characters indicating high rank. The presence of several such characters in the Urticales is not an indication of particular advance over other groups. Nor is the presence of zygomorphism, for example, an indication of relationship with another group in which the same feature is present. That the Urticales are related to one of those plexuses of the angiosperms possessing types of zygomorphism, namely: that culminating in the Monocotyledons, that of the Rosales in the Polypetalae, and that of the Tubiflorae in the Sympetalae, can receive no support. It does seem that a relationship more nearly correct may be discovered for the Urticales by considering the characters possessed by them that indicate primitiveness rather than those that indicate specialization, namely: many organs, non-cyclic conditions, and preponderance of woody forms. The one order of the angiosperms possessing these characters is the Ranales. The Ranales have not suffered reduction to any degree comparable with that of the Urticales. Floral anatomy of the members of the families of the Ranales may reveal important characters that macroscopic study cannot reach. However, the Urticales appear, when viewed from the standpoint of their primitive characters, to be parallel with the Ranales. The latter possesses a tendency to the pentamerous condition, and both orders possess a tendency to an unicarpellate condition. That the Urticales and Ranales are descendants from the same protoangiospermous plexus seems likely. But, since the flowers of the Urticales are greatly reduced in each set of organs, as the floral anatomy described above indicates, the Urticales are on a higher level than the Ranales.

A feature of the Urticales that has caused them to be looked upon as very primitive plants among the angiosperms is the "orthotropous" ovule. This type of ovule has been regarded as the most primitive since it is apparently the common type appearing in those families classified as lowest in the Polypetalae. In the most highly reduced members of the Urticales, numbering about half the species of the order, the ovule is "basal" or "orthotropous." Anatomical work reveals, however, that the ovule has become basal, as previously described, by a sinking or a sliding down from a pendulous position and that in this process the anatropous ovule has become erect. Thus the Urticaceae show a phyletic origin of the orthotropous ovule from an anatropous, pendulous, or lateral type, as Welsford and Benson (22) consider is the case in *Juglans regia* and related plants, basing their evidence also on anatomical study. The orthotropous ovule in this group, therefore, is not primitive. The floral anatomy of Bentham's Incompletae (15 orders), in which the ovule, with few exceptions, is basal, is an inviting line of research. In determining the phyletic relationship of the Urticales, therefore, it is the pendulous or lateral anatropous ovule that must be considered and not the erect basal ovule.

A consideration of the vascular supply to the ovule, as described in the species studied, may indicate that the Urticales are not a natural order. Three types of vascular supply to the ovule were found, but these are all the results of the greatly reduced condition of the flowers. The common type, as found in Ulmus, Morus, Maclura, and Urtica, representatives of the three families of the order, is an ovule supply which is the result of the fusion of two lateral strands from the floral axis. The second type is found in Celtis and in Laportea, where the ovule supply is a branch from the anterior carpellary strand. The third type is in Humulus and in Cannabis, where the ovule supply is a continuation of a single strand from the pedicel.

The first two suggest a foliar origin for the ovules. The two lateral strands passing to the ovule are two lateral basal veins arising with the midvein of the anterior carpel. This condition strongly suggests the carpel to be a foliar organ with palmate venation. Possibly the ancestral condition was that of a carpel with several ovules, two at least, one borne on each of these basal lateral veins; but through coalescence and reduction the two veins conjoined and one ovule was crowded out. It is likely that other lateral veins of the dorsal carpellary bundle above the two existing bearing ovules have disappeared through the same processes. The same thing seems to have happened in the posterior carpel of Ulmus. An abortive ovulary branch of the placental strand, in which are incorporated the abortive lateral strands (basal veins) in the anterior and posterior carpels, is present just opposite the branch passing into the ovule (Pl. XVI, fig. 19, i^1; Pl. I, fig. 3, i^1). The ovule belonging with this strand is occasionally present in Ulmus and in Morus (Baillon, 1; Engler, 9). The strand leading to the ovule in Humulus and in Cannabis arises deep in the pedicel. It is posterior and opposite to the strand that passes into the enveloping bract (Pl. XX, fig. 2, *o*, *a*), and is the largest of the four strands in the pedicel. The single pedicellar strand to the ovule and the phenomenon present there are due undoubtedly to the greatly reduced state of the flowers, described previously (p. 402), which has altered the ovule supply to a single strand. In the anterior carpel the lateral carpellary veins have disappeared and the midrib is small, undoubtedly because of the development of the large bract. The same bundles in the posterior carpel have fused into one strand passing to the single ovule. The ovule and the ovule supply, therefore, indicate a natural order for the Ulmaceae, Moraceae, and Urticaceae. When the ovule of the Urticales is taken into consideration to determine the likely relationship of the order, the type of ovule as found in Ulmaceae must be used. That type is the anatropous, pendulous, or lateral ovule, which is the primitive type in the Urticales. The partial basal or basal-erect ovules are the result of reduction as the comparative anatomical studies previously described indicate.

The accepted relationship of these three families on the part of taxonomists is supported by this study of floral anatomy. The Urticaceae are higher than the Moraceae, *i.e.*, they are more reduced in carpels and in perianth. The Moraceae are higher than the Ulmaceae, *i.e.*, they are more reduced in number of stamens and in perianth parts. Also, the generic relationships are indicated by this anatomical study. In the Urticaceae, Laportea and

Boehmeria are higher than Urtica, and Boehmeria is higher than Laportea. In Moraceae, Maclura is higher than Morus; and in the Ulmaceae, Celtis is higher than Ulmus. In the genus Ulmus the result of these anatomical studies places species in the same groups in which they have been placed by taxonomists. *U. americana* and *U. racemosa* come in one group, and *U. fulva*, *U. scabra*, and *U. campestris* come together in another group.

The natural position of the Urticales has been a debated subject. The common practice has been to place them in association with the Amentiferae. Jussieu, de Candolle, Endlicher, Bentham, Hooker, Engler, and Gray have assisted in establishing this arrangement. The Amentiferae, however, are coming to be looked upon as reduced rather than as primitive forms. Weddell (21), in 1840, associated the Urticales with Tiliaceae and Malvaceae, etc. One of the features that influenced him in making such a decision was the presence of "bast fibers"; but on the same feature, a relationship can be established with Thymeliales, which possesses several similar floral structures. Lindley (13), in 1845, placed the Ulmaceae singly in the Rhamnales. Bessey (3) and Hallier (12), in 1905, placed the Ulmaceae, Moraceae, and Urticaceae in the Malvales, as Weddell had done sixty years before. The last suggestion has received much favorable consideration from many taxonomists. The writer's anatomical studies in these suggested affinitives have not progressed far enough to warrant any conclusive statement.

The floral anatomy of the species of Ulmus reveals a feature that should be discussed at this time, namely: the staminal cylinder as described on page 389 (Pl. XVI, figs. 16, 17, *c*). This may be considered homologous with the staminal tube of the Malvaceae. Yet, the cohesion of filaments is a character occurring in the Parietales, Geraniales, and in other small groups, and is a striking character in the Papilionaceae. The Malvales, as delimited by Engler (9), show the tendency to chorisis. Reduction, which is opposed to chorisis, is conspicuous in the Ulmaceae, Moraceae, and Urticaceae. However, it may be possible to accept a natural order exhibiting two such diverse processes.

There is the danger of placing the Urticales higher than they should be, due to the greatly reduced flower condition; the caution from Engler (10) in this regard has already been stated. Such an error can possibly be avoided by considering the characters of the order that indicate primitiveness, namely, many organs, non-cyclic condition, and preponderance of woody forms. On the other hand, zygomorphism and reduction are present in the order not as tendencies but as critical characters, *i.e.*, the characters present throughout the order. Therefore, these tendencies must be present in their nearest relatives, or were present in their immediate ancestors. It is doubtful that their ancestors were wind-pollinated. The progenitors of the Urticales are not in existence today. Considering their primitive characters, they are in a distinct line of descent from a protoangiospermous plexus from which also descended the Ranalian line. The Urticales have advanced parallel with the Ranalian stock to a high degree of specialization, namely, zygomorphy. Accompanying this specialization, or following it, the Urticales show great reduction in all parts of the flower. The result has been a group of plants combining characters belonging to primitive and to recent types, a combination which makes them a generalized rather than a specialized group from which no descendants seem to have arisen.

Summary and Conclusions

1. The anatomy of the flowers of the Urticales reveals a number of features extending throughout the order, which are not appreciable from a macroscopic investigation.

    a. Ulmus, the primitive genus, shows evidence of suppression of a whorl of stamens and of one of perianth parts. The existing stamens are fused with the gamophyllous perianth. The parts of these whorls are somewhat spiral in arrangement and very inconstant in number.

    b. The bicarpellate condition has been derived from a polycarpellate condition as evidenced by the presence of vascular supply to suppressed carpels. Also, vestigial bundles indicate that the bicarpellate gynoecium is becoming unicarpellate by the suppression of one carpel.

    c. The perianth parts are reduced in number by abortion, suppression, and fusion; in some cases the inner whorl has entirely disappeared, in others vestiges of its vascular supply remain. In some forms the inner and outer whorls are fused and occur as one whorl.

    d. Zygomorphy is a conspicuous character of all species studied; evidence of it is not only found externally but appears also on microscopic study of transverse sections of the flowers.

    e. Palmate venation, if no longer present in the foliage, is still present in the perianth parts in some forms.

    f. The ovules are foliar organs. The orthotropous ovule in the higher members of the order has come to its basal, erect position by a sinking from an apical or lateral position of the anatropous ovule in the primitive members. The "cauline" ovule in the Urticales is apparently such due to reduction. All "cauline" ovules may possibly be simply the result of the same process.

    g. The vascular supply to the uniovulate ovary suggests a polyovulate ancestry.

    h. Accompanying coalescence and adnation, the flowers have been greatly reduced in all floral organs.

2. In plant organs suffering reduction the vascular system disappears in advance of the organs, or persists as abortive bundles after the organs have disappeared.

3. The combination of primitive and specialized characters makes the Urticales a generalized group.

4. The Urticales are probably not far removed from primitive entomophilous ancestors.

5. Floral anatomy emphasizes the idea that the Urticales are a natural order which is made up of three natural families as classified by Engler.

6. The natural position of the Ulmaceae, Moraceae, and Urticaceae is at the culmination of a distinct line of descent from a protoangiospermous plexus from which also the Ranalian line descended.

The writer wishes to express his gratitude to Dr. A. J. Eames, to whom he is indebted for this problem as well as for advice and assistance while the work was in progress.

Department of Botany,
College of Agriculture,
Cornell University,
Ithaca, N. Y.

LITERATURE CITED

1. **Baillon, H.** Histoire des plantes. 7 vols. Paris, 1866–1880.
2. **Bentham, G., and Hooker, J. D.** Genera plantarum. 3 vols. London, 1862–1883.
3. **Bessey, C. E.** The phylogenetic taxonomy of flowering plants. Ann. Mo. Bot. Gard. **2**: 109–164. 1915.
4. **Bower, F. O.** The origin of a land flora. London, 1908.
5. **Darwin, C.** On the various contrivances by which British and foreign orchids are fertilized by insects, and on the good effects of intercrossing. London, 1862.
6. **de Candolle, A. P.** Théorie élementaire de botanique. Paris, 1813.
7. **Eames, A. J.** On the origin of the herbaceous type in the angiosperms. Annals of Bot. **25**: 215–224. 1911.
8. **Eichler, A. W.** Blüthendiagramme. Vol. 2. Leipzig, 1878.
9. **Engler, A.** Die natürlichen Pflanzenfamilien. Leipzig, 1897–1915.
10. **Engler, A., and Gilg, E.** Syllabus der Pflanzenfamilien. 7th ed. Berlin, 1912.
11. **Gérard, R.** Sur l'homologie et le diagramme des Orchidées. Ann. Sci. Nat. Bot. VI, **8**: 213–247. 1879.
12. **Hallier, H.** Provisional scheme of the natural (phylogenetic) system of flowering plants. New Phytol. **4**: 151–162. 1905.
13. **Lindley, J.** The vegetable kingdom. London, 1853.
14. **Payer, J. B.** Traité d'organogénie végétale comparée de la fleur. Paris, 1857.
15. **Planchon, J. E.** Ulmaceae. In de Candolle's "Prodromus" **17**: 151–210. 1873.
16. **Pritchard, F. J.** Change of sex in hemp. Jour. Hered. **7**: 325–329. 1916.
17. **Robertson, C.** The structure of the flowers and the mode of pollination of the primitive angiosperms. Bot. Gaz. **37**: 294–298. 1904.
18. **Sinnott, E. W., and Bailey, I. W.** The origin and dispersal of herbaceous angiosperms. Annals of Bot. **28**: 547–600. 1914.
19. **Sinnott, E. W., and Bailey, I. W.** Foliar evidence as to the ancestry and early climatic environment of the angiosperms. Amer. Jour. Bot. **2**: 1–22. 1915.
20. **Van Tieghem, P.** Recherches sur la structure du pistil. Ann. Sci. Nat. Bot. V, **9**: 153–226. 1868.
21. **Weddell, H. A.** Urticaceae. In de Candolle's "Prodromus" **16**: 32–235^{64}. 1869.
22. **Welsford, E. J., and Benson, M.** The morphology of the ovule and female flower of *Juglans regia* and of a few allied genera. Annals of Bot. **23**: 623–633. 1909.

23. **Wernham, H. F.** Floral evolution: with particular reference to the sympetalous dicotyledons. IX. New Phytol. **11**: 373–397. 1912.
24. **Zinger, N.** Beiträge zur Kenntniss der weiblichen Blüthen und Inflorescenzen bei Cannabineen. Flora **85**: 189–253. 1898.

EXPLANATION OF PLATES

The figures in the plates are greatly enlarged. The flower sections range from 0.5 mm. to 3 mm. in diameter, or 3 mm. x 5 mm. in dimension.

PLATE XV

Ulmus americana

FIG. 1. Habit sketch of flower with 8 perianth lobes and 8 stamens.

FIG. 2. Portion of perianth showing small inner lobe (*a*) and vascular supply to lobes.

FIG. 3. Longitudinal section of flower in median posterior-anterior plane. Origin of vascular supply (*b*) to perianth (*p*) and to stamens (*s*); 1, bundle to anterior carpel; 1^1, bundle to posterior carpel; *ov*, to placenta; *i*, to ovule in anterior carpel; i^1, the abortive bundle to suppressed ovule in posterior carpel; o^1 and o^2, placental branches passing through the inner faces of the styles; d^6, abortive bundle alternating with those to the stamens.

FIG. 4. Longitudinal section of flower perpendicular to the plane of that in figure 3; d^2, d^4, abortive bundles alternating with stamen bundles; *e*, abortive bundle in the carpel supply.

FIG. 5. Transverse section through pedicel; *st*, the stele.

FIGS. 6–15. Transverse sections through a flower, 40, 50, 30, and (figs. 9–15) 20 microns apart respectively. Posterior trace passes off and separates into traces to perianth lobe (*m*) and stamen (*s*). Other traces pass off successively toward the anterior side, m^1, n^1, . . . m^7, n^7, and each separates as does the posterior trace. Stamen trace n^2 aborts 70 microns above its origin; d^1 . . . d^6, abortive bundles of vascular supply continuing above and alternating with that to the stamens; *c*, staminal cylinder; *t*, traces continuing into the pistil.

PLATE XVI

FIGS. 16–18. Transverse sections of flower; *m* . . . m^7 and *n* . . . n^7, bundles to perianth and stamens respectively. The posterior (*m*) and anterior (m^7) bundles separate each into 3 strands; 1, to posterior, 1^1, to anterior carpel; strands *o* come together making $o + o$ of the placental vascular system.

FIGS. 19–21. Transverse sections through upper part of pistil; 1 and 1^1, dorsal bundles to carpels; *i*, to ovule; i^1, abortive bundle corresponding to *i*; o^1 and o^2, inner lateral bundles of styles.

FIG. 22. Section through a flower at level of stalk of pistil, an 8-merous flower.

FIG. 23. Section through a flower with 7 perianth lobes and 6 stamens.

FIG. 24. Section through a 9-merous flower.

FIG. 25. Section through a flower having a stamen (*nk*) with no accompanying perianth lobe.

PLATE XVII

Ulmus fulva

FIG. 1. Median longitudinal posterior-anterior section; supply to stamens originates some distance (6) above that to perianth; *o*, the placental strand which branches like that in *U. americana* (Pl. XV, fig. 3); *d*, abortive bundles.

FIG. 2. Transverse section through lower part of flower; *p*, perianth; *s*, stamens; *d*, abortive strands; s^1, abortive (lignified) strand to suppressed stamen; *t*, continuation of floral axis above the stamen supply.

Ulmus racemosa

FIG. 3. Median longitudinal posterior-anterior section of flower; *b*, origin of perianth (*p*) and stamen (*s*) strands; *o*, placental supply.

FIG. 4. Transverse section through lower part of flower; *m* . . . m^7 and *n* . . . n^7, strands to perianth and to stamens respectively; *d* and d^1, abortive bundles.

Ulmus campestris

FIG. 5. Median longitudinal posterior-anterior section; bundles to stamens (*s*) originate above (*b*) those to perianth (*p*).

FIGS. 6, 7. Various lobing of perianth; 1 . . . 4 are strands to main lobes; *a* and *b*, branches or separations from the main strands; *s*, stamen position.

Ulmus scabra

FIG. 8. Longitudinal lateral section of flower; *b*, space between origin of stamen and perianth bundles; *x*, x^2, abortive bundles of the perianth; *d*, abortive bundles above those to stamens; *o*, the placental supply.

FIGS. 9, 10. Transverse section of flower; *m* . . . m^5 and *n* . . . n^5, bundles to perianth lobes and stamens respectively; *x*, x^1, x^2, abortive bundles of perianth; *d*, abortive bundles alternating with stamen bundles; *t*, continuation of floral axis; 1, 1^1, 1^2, dorsal bundles of 3 carpels; *o*, the placental supply.

PLATE XVIII

FIGS. 1, 2. Transverse sections above those of figures 9 and 10, Plate XVII; lettering the same. Perianth bundles separate into 2 to 4 strands.

Celtis occidentalis

FIG. 3. Longitudinal section of flower; *b*, trace from stele of pedicel which separates into perianth (*p*) and stamen (*s*) strands; *o*, placental supply arises or separates from anterior carpel (1^1) supply.

FIGS. 4–12. Transverse sections of flowers; in lower part of flower, stele is prominent in anterior and posterior regions; strands to anterior part of flower lead off, perianth (*m*) and stamen (*n*); 1, 1^1, dorsal bundles of carpels; *o*, bundle of placental supply; o^1 and o^2, lateral strands of the pistil.

FIG. 13. Transverse section at base of styles, o^1 and o^2 not present.

PLATE XIX

Morus alba, pistillate

1, anterior, and 1^1, posterior carpel bundles; *p*, posterior and anterior, and p^1, lateral sepals; *o*, placental supply.

FIG. 1. Median longitudinal section of flower in posterior-anterior plane.

FIGS. 2, 3. Transverse section through pedicel.

FIGS. 4–9. Transverse sections of a flower; $o + o$, the union of two placental strands (as in Ulmus).

FIG. 10. Longitudinal section of a staminate flower; *b*, strand composed of stamen and perianth supply; 1, 1^1, abortive carpel bundles.

Maclura pomifera

FIGS. 11, 12. Transverse sections through base of pistillate flower within the inflorescence axis; 1, 1^1, anterior and posterior carpel supply; *p*, perianth; *o*, placental supply.

FIGS. 13–17. Flowers becoming distinct as well as the parts of each flower; *n*, abortive bundles in the perianth parts.

FIGS. 18, 19. Longitudinal median anterior-posterior section of pistillate and staminate flowers respectively.

PLATE XX

Cannabis sativa, pistillate

br, bract; *s*, bundle to bract; *p*, perianth; 1, 1^1, traces to carpels; *s*, stamen; *o*, bundle to placenta; *x*, abortive bundles.

FIG. 1. Habit sketch of flower; *p*, cup-like perianth.

FIGS. 2–8. Transverse sections of flower. Origin and freeing of bract and perianth; bundles to carpels and to ovule.

Humulus Lupulus, pistillate

(Lettering as for Cannabis)

FIG. 9. Longitudinal median posterior-anterior section of flower.

FIGS. 10–12. Origin of bract, presence of abortive bundles.

FIGS. 13–18. Origin of perianth bundles numbered in the order of their appearance. Two carpel bundles continue into the styles.

PLATE XXI

Urtica gracilis, pistillate

1, 1^1, anterior and posterior carpel supplies; *o*, *o* + *o*, placental supply; *m*, posterior and anterior, and *n*, lateral perianth parts.

FIG. 1. Longitudinal median anterior-posterior section of flower.

FIG. 2. Transverse section of pedicel.

FIGS. 3–13. Transverse sections of flower; origin and the freeing of floral parts; supply to ovule.

PLATE XXII

Boehmeria cylindrica

(Lettering as for Urtica)

FIG. 1. Longitudinal median anterior-posterior section of pistillate flower; *o*, bundle to ovule.

FIG. 2. Transverse section of pedicel.

FIGS. 3–7. Transverse sections of pistillate flower; origin of floral organs and same becoming distinct.

FIG. 8. Transverse section of style.

FIGS. 9–13. Sections of staminate flower; 1, 1^1, abortive bundles to abortive pistil.

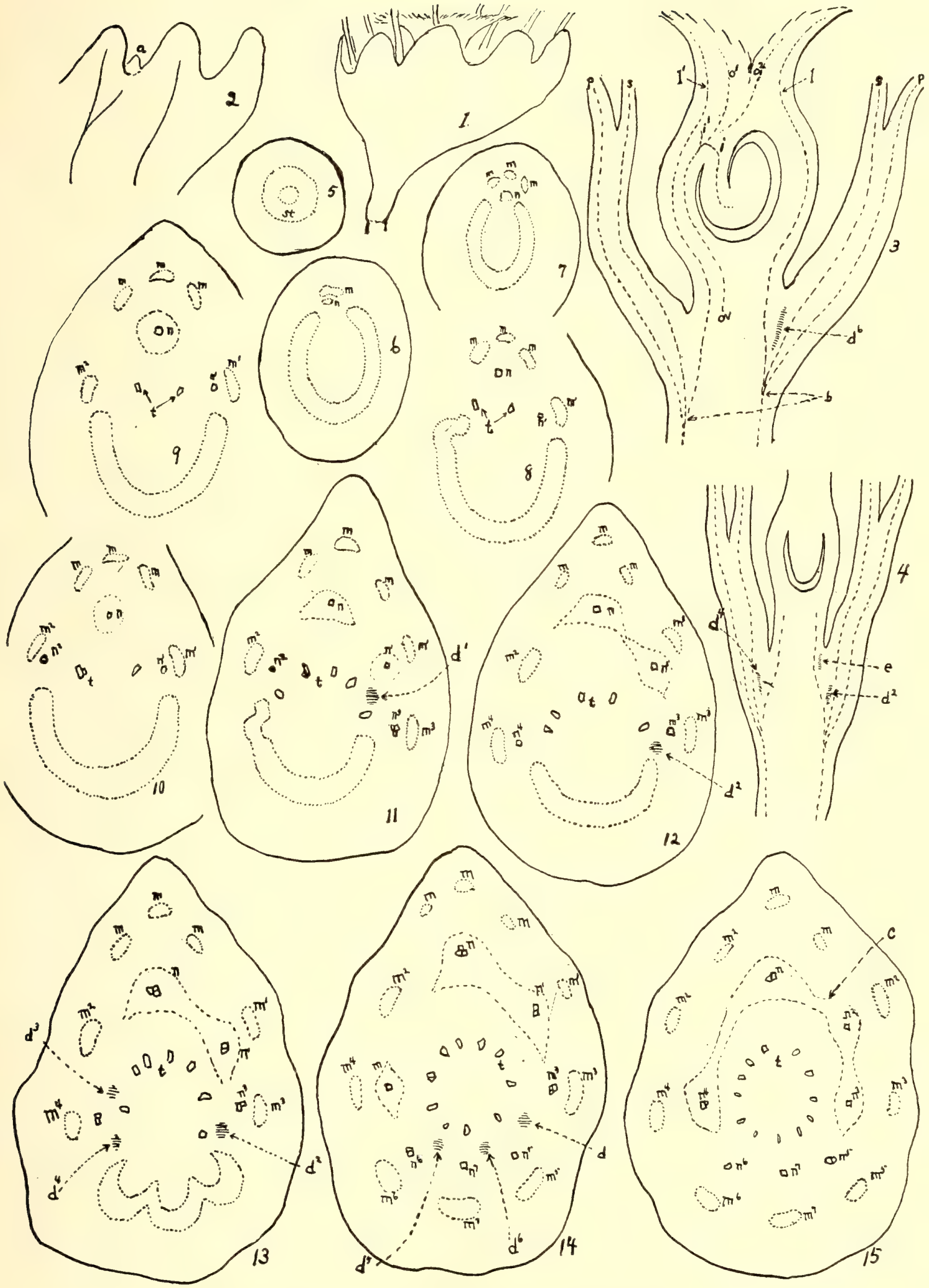
Laportea canadensis

(Lettering as for Urtica and Boehmeria)

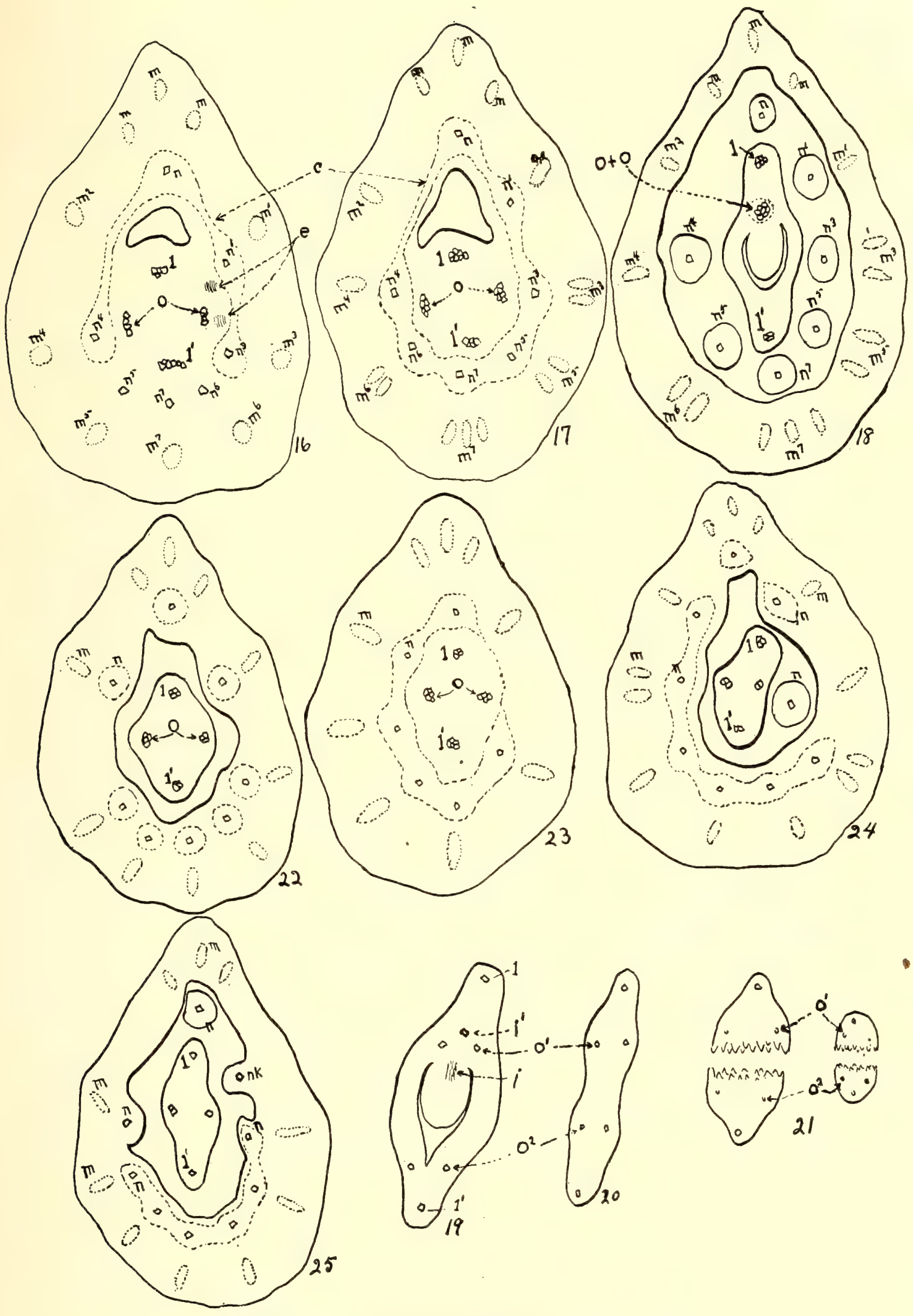
FIG. 14. Longitudinal median posterior-anterior section of pistillate flower; *f*, funiculus.

FIG. 15. Transverse section of pedicel.

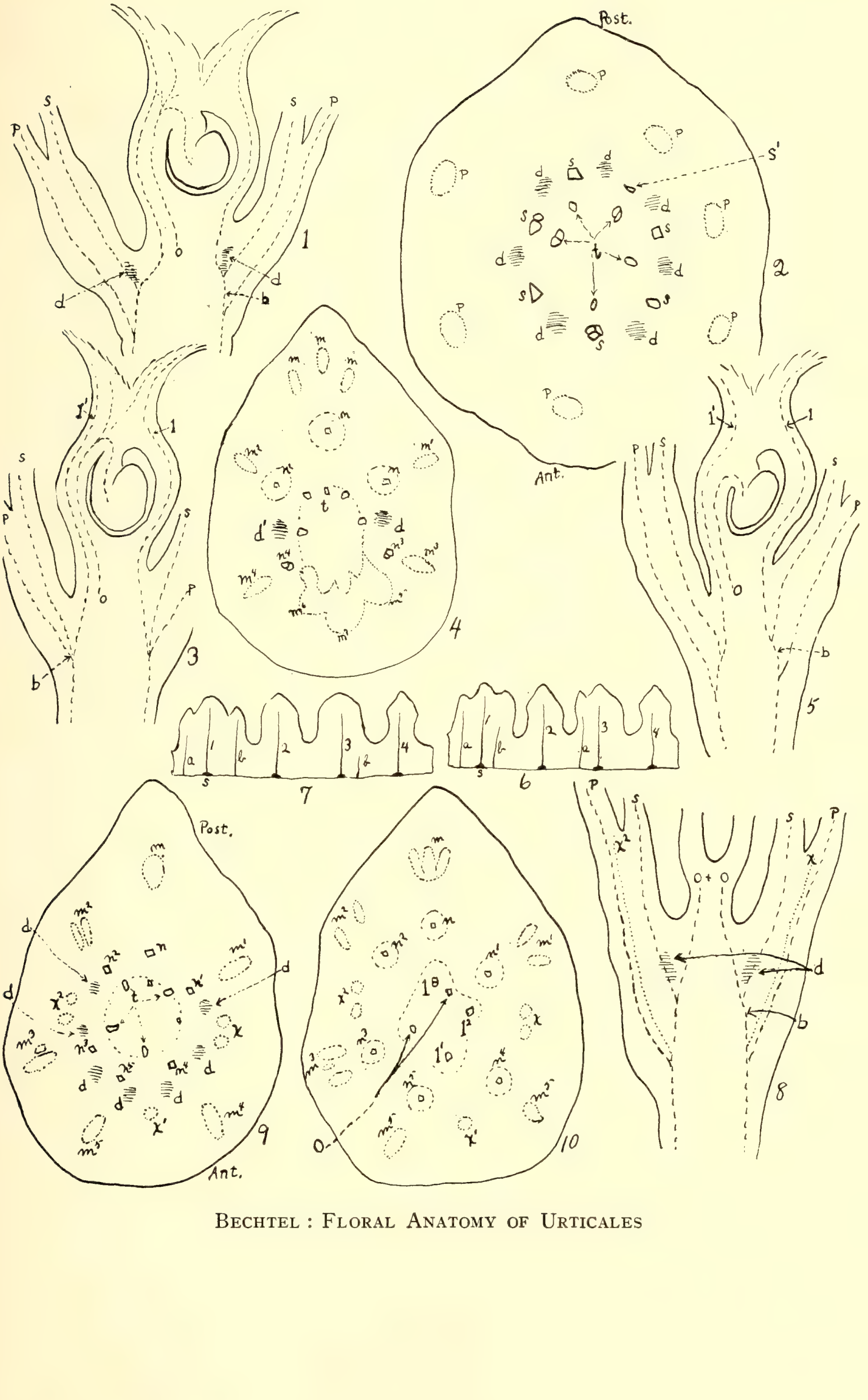
FIGS. 16–21. Transverse sections of pistillate flower; origin of floral parts and the same becoming distinct; ovule supply separating from anterior carpel supply; 1, abortive anterior carpel bundle; m^1, posterior sepal has its vascular supply suppressed.


1
2
a
3
p
s
l'
l
ov
d⁶
b
4
d⁴
e
d²
5
st
6
7
8
9
10
11
12
13
14
15
m
n
m'
m²
m³
m⁴
m⁵
m⁶
m⁷
n'
n²
n³
n⁴
n⁵
n⁶
n⁷
t
d'
d³
d⁵
d
c

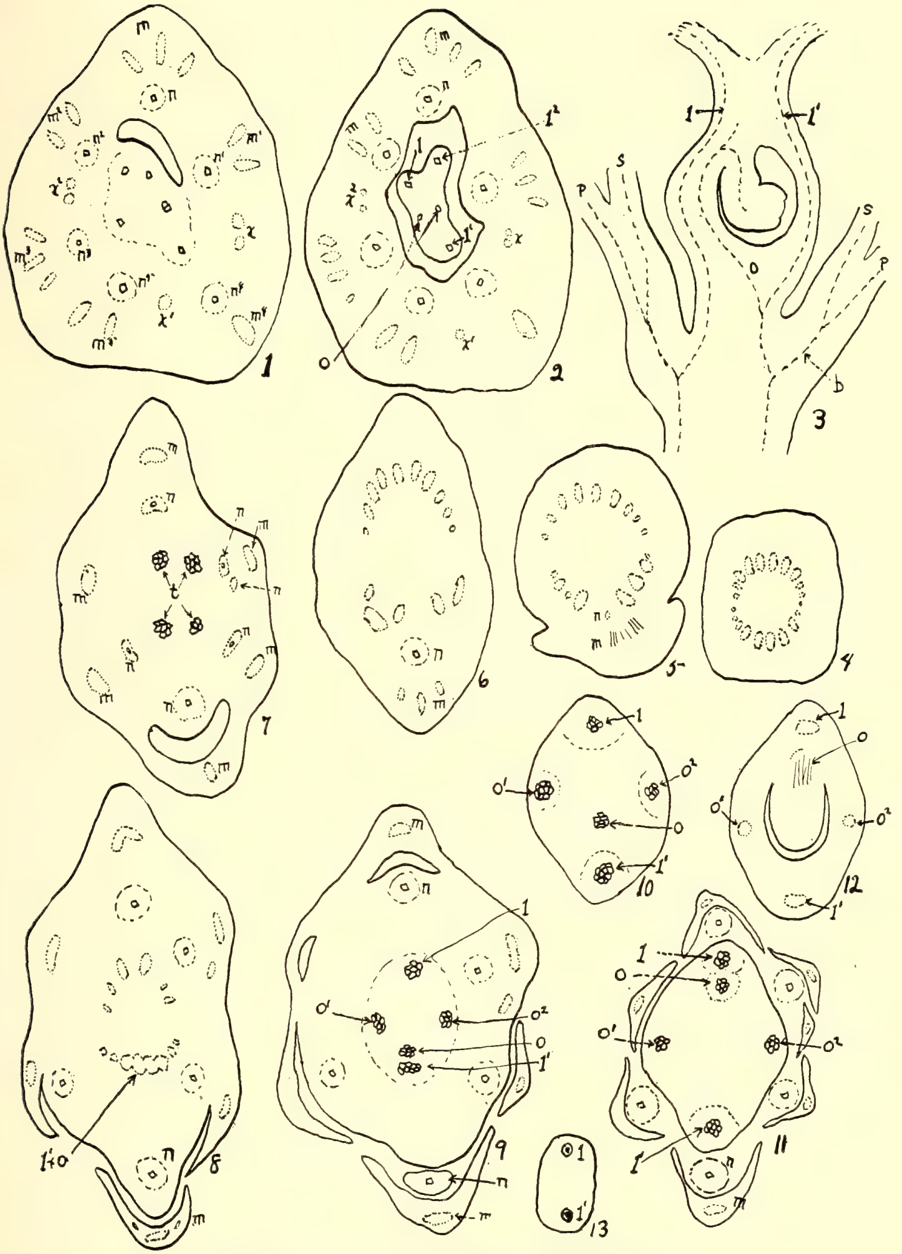
BECHTEL : FLORAL ANATOMY OF URTICALES


m
m²
m³
m⁴
m⁵
m⁶
m⁷
n
n³
n⁴
n⁵
n⁶
n⁷
c
e
o
1
1'
o+o
nk
i
o'
o²
16
17
18
19
20
21
22
23
24
25

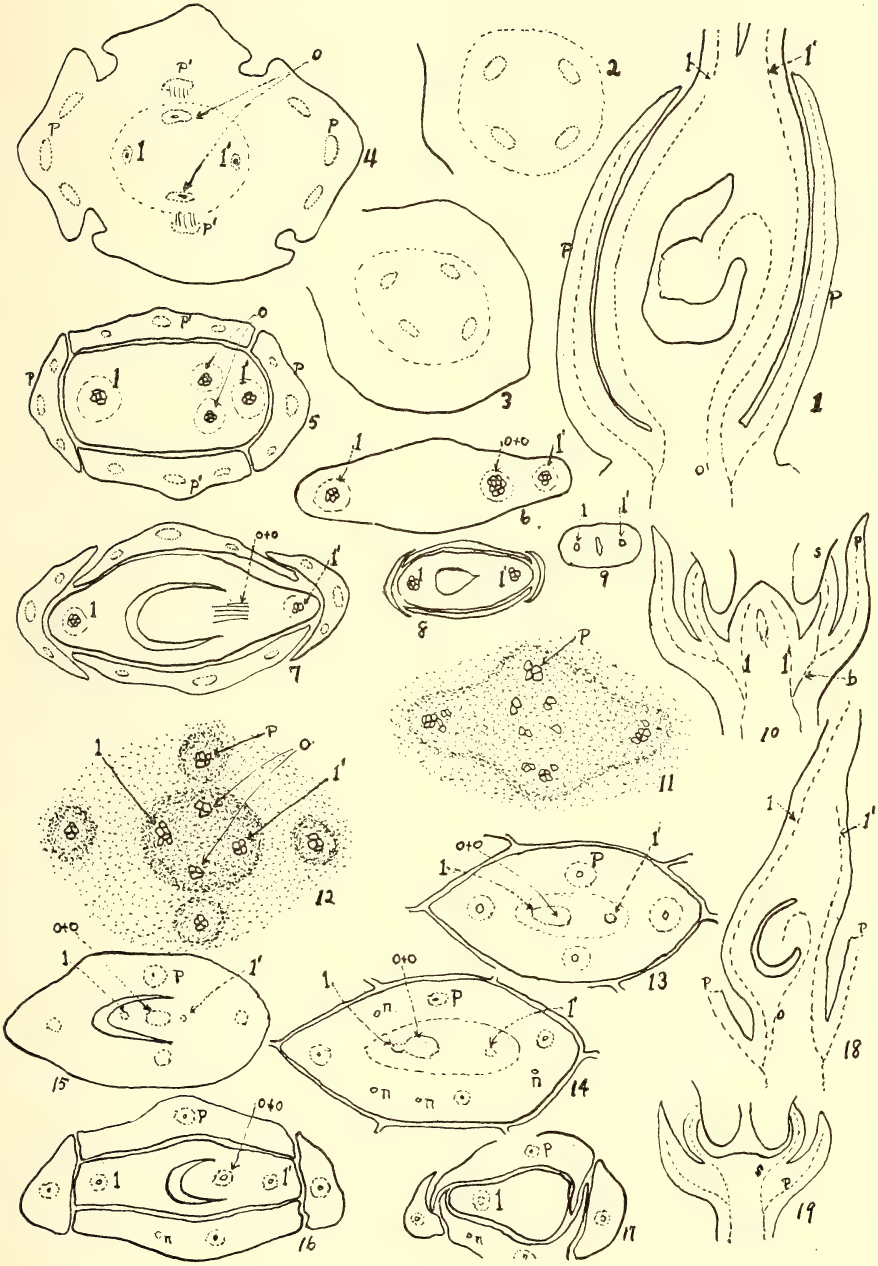
BECHTEL: FLORAL ANATOMY OF URTICALES


s
p
o
d
b
1
Post.
Ant.
2
s'
t
3
1'
4
m
m'
m²
m³
m⁴
n'
n³
n⁴
d'
5
7
6
a
8
x
x²
o + o
9
x'
n²
m⁵
10
1²
1³
1'

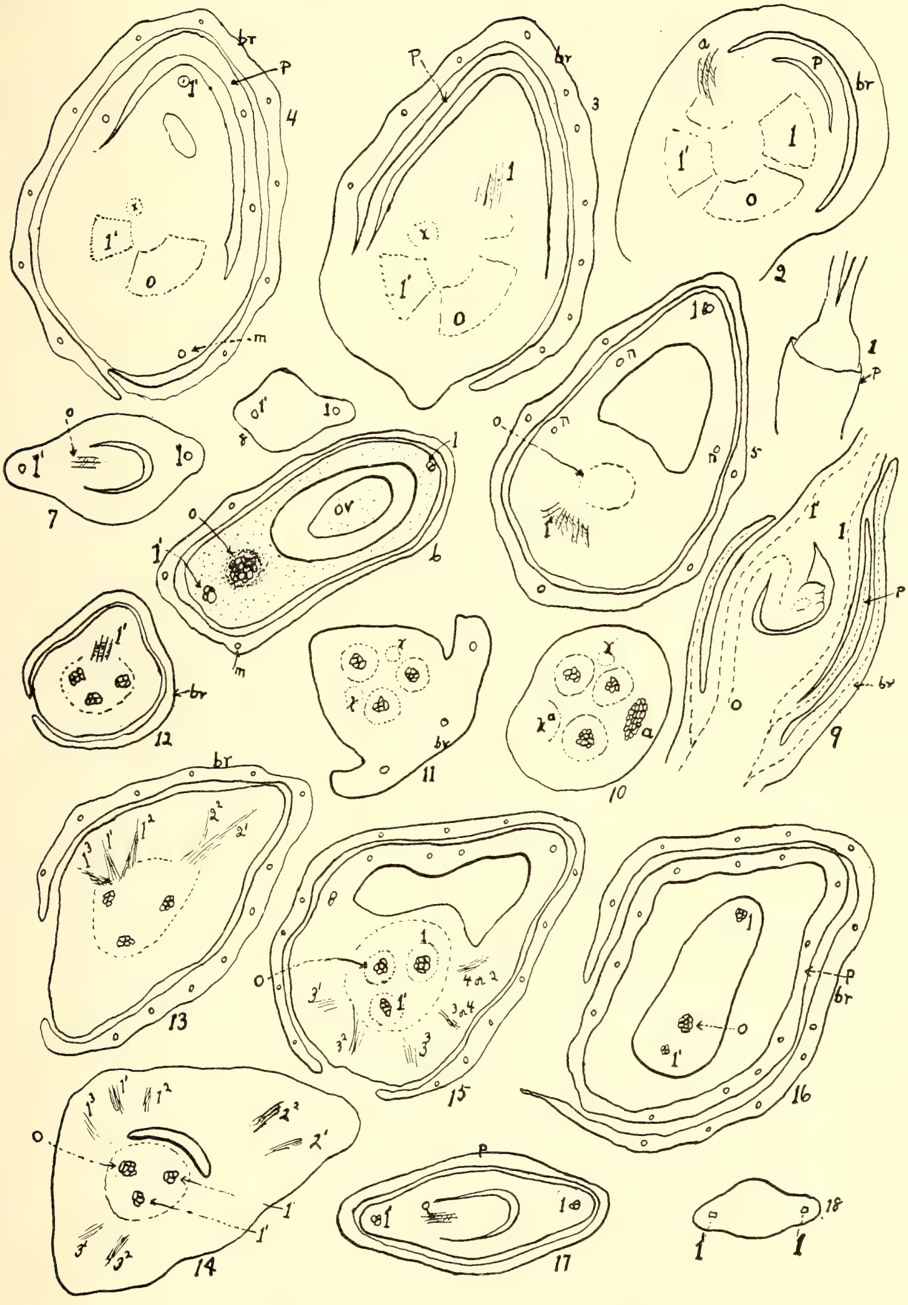
BECHTEL : FLORAL ANATOMY OF URTICALES


1
2
3
4
5
6
7
8
9
10
11
12
13
m
n
x
o
1
1'
s
p
b
t
o'
o²
1+o

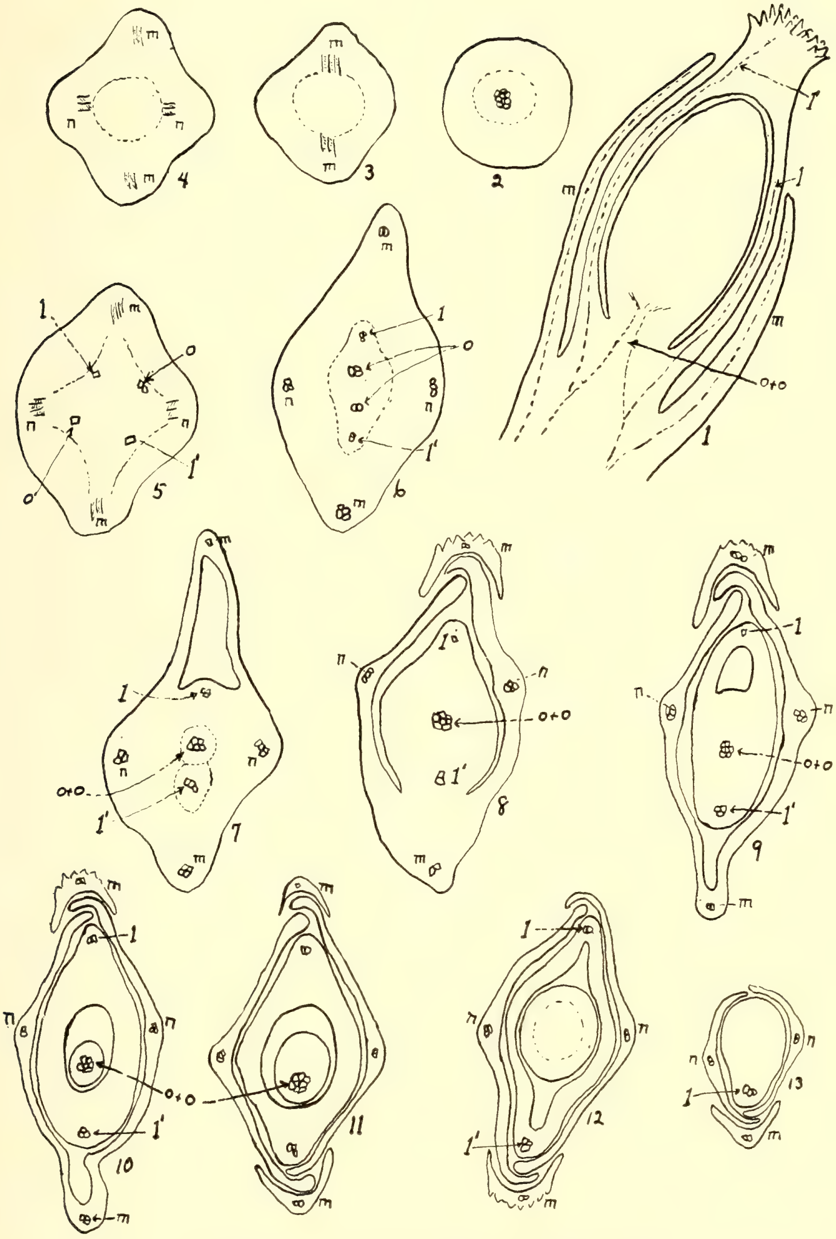
BECHTEL: FLORAL ANATOMY OF URTICALES


o
p'
p
1
1'
p'
4
2
1
1'
p
p
1
3
o
p'
p
p
1
1'
5
p'
1
o+o
1'
6
1
1'
9
o+o
1'
1
7
1
1'
8
s
p
1
1'
b
10
p
11
1
p
o
1'
12
o+o
1-
p
1'
13
1
1'
o
p
18
o+o
1
p
1'
15
o+o
1
p
on
1'
n
14
on
on
o+o
p
1
1'
16
cn
p
1
on
17
s
p
19

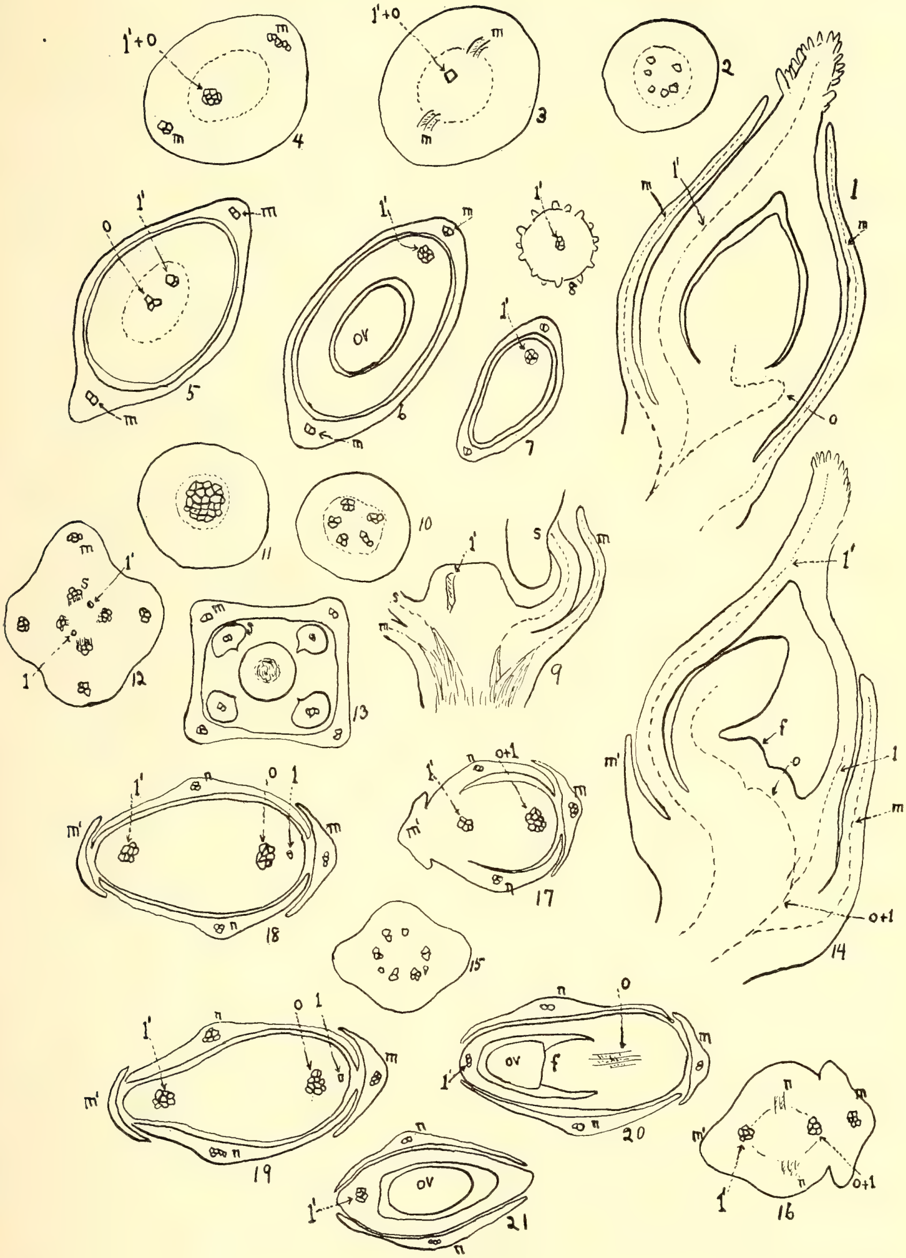
BECHTEL: FLORAL ANATOMY OF URTICALES


4
br
P
1'
x
1'
o
m
3
br
P
1
x
1'
o
2
a
P
br
1
1'
o
1
P
1b
n
n
n
o
1'
5
7
o
1'
1o
8
o1'
1o
6
1
ov
o
1'
m
12
1'
br
11
x
x
br
10
x
x^a
a
9
1'
1
P
br
o
13
br
1^3
1'
1^2
2^2
2'
15
1
o
1'
3'
3^2
3^3
4 or 2
3 or 4
16
1
P
br
o
1'
14
1^3
1'
1^2
2^2
2'
o
1'
1'
3'
3^2
17
P
1
1
18
1
1

BECHTEL: FLORAL ANATOMY OF URTICALES


4
3
2
1
5
6
7
8
9
10
11
12
13
m
n
1
1'
o
o+o

BECHTEL : FLORAL ANATOMY OF URTICALES


1'+o
m
4
1'+o
m
3
2
1
1'
o
5
6
ov
7
8
11
10
12
s
13
9
14
f
o+1
m'
n
17
18
15
19
20
ov
16
21

BECHTEL : FLORAL ANATOMY OF URTICALES

GENETIC EVIDENCE OF ABERRANT CHROMOSOME BEHAVIOR IN MAIZE ENDOSPERM[1]

R. A. Emerson

(Received for publication February 26, 1921)

The occasional appearance of a maize seed, the endosperm of which is in part colored and in part colorless or in part starchy and in part sugary, has long been known, and much speculation has been indulged in by geneticists in attempts to account for the phenomenon. Some years ago the writer (Emerson, 1915) reviewed the hypotheses that had been previously offered as possible explanations of such seeds and suggested the further hypothesis of somatic mutation, a suggestion that has been repeated, apparently independently, by J. L. Collins (1919). It was noted also that irregular chromosome behavior might possibly be concerned. In a later paper (Emerson, 1918) numerous cases of anomalous endosperm development were reported and discussed in relation to the hypotheses of somatic mutation and of aberrant chromosome behavior. It was pointed out that the facts then at hand could be accounted for equally well by either of the two hypotheses, and the kind of evidence necessary for a crucial comparison of the two was noted.

In the latter paper evidence was presented that tended to prove that aberrant seeds are not produced (1) when the dominant endosperm factor concerned, for example the aleurone-color factor *C*, is homozygous and therefore triplex, *C C C*, or (2) when the dominant factor is brought into the cross by the female parent and its recessive allelomorph by the male parent, *C C c*, but only (3) when the dominant factor is contributed by the male alone, *c c C*. In the case of either *C C C* or *C C c*, a single mutation from the dominant to its recessive allelomorph could result only in *C C c* or *C c c*, respectively, and the aleurone would still be colored and no apparent anomaly would result. To produce colorless aleurone, *c c c*, two mutations in case of *C C c* and three in case of *C C C* must occur simultaneously or successively in the endosperm of the same seed—a chance so small that it might well be disregarded. It was noted that a single dominant mutation from *c* to *C* should change colorless, *c c c*, to colored, *C c c*, aleurone, but the relative infrequency of dominant mutations was thought to account for the lack of observed aberrant seeds in homozygous colorless types.

Similarly, it was noted that if a single non-disjunction of the chromosome carrying *C* or *c* occurred, it could not result in a visible change in aleurone color in case of such genotypes as *C C C*, *C C c*, or *c c c*, but only with *c c C*. In the latter case, if the chromosome carrying *C* failed to divide or if the two halves failed to separate after division, one of the resulting daughter nuclei would be *c c C* or *c c C C* (colored) and the other *c c* (colorless). Thus both somatic mutation and chromosome non-disjunction might readily account for the observed cases of aberrant endosperm, and neither mutation nor non-disjunction could reasonably be expected to cause such an anomaly in genotypes where it has never been observed.

---

[1] Paper No. 86, Department of Plant Breeding, Cornell University, Ithaca, New York.

It was pointed out in the writer's 1918 paper that crucial evidence in support of one or other of these hypotheses might be obtained only from crosses in which linked aleurone and endosperm factors are simultaneously involved. It was known that the aleurone factor pair *C c* is thus linked with waxy endosperm, *Wx wx* (Bregger, 1918; Kempton, 1919), but no aberrant seeds positively known to involve both these factor pairs were available. The writer was not unaware of G. N. Collins's case (1913) involving the aleurone factor pair *I i* with *Wx wx*, but the linkage relations of these factors were not known. It has since been shown by Hutchison (1921) that the factor pair *I i* is closely linked with a factor pair for shrunken endosperm, *Sh sh*, which in turn is linked with *C c* and *Wx wx*. The linkage group as at present known, therefore, is made up of the pairs *C c*, *I i*, *Sh sh*, and *Wx wx*. Consequently, in accordance with the chromosome hypothesis, all these factor pairs are assumed to lie in one pair of homologous chromosomes.

Assuming, then, that *C* and *Wx* lie in the same chromosome, it can readily be seen how a crucial test of the somatic-mutation and the chromosome-non-disjunction hypotheses is afforded by appropriate crosses. If the female parent of a cross be colorless and waxy, *c wx*, and the male parent be colored and corneous, *C Wx*, the resulting endosperm will be *c c C wx wx Wx*, and the three homologous chromosomes carrying these genes in the "fecundated" endosperm nucleus will be as follows:

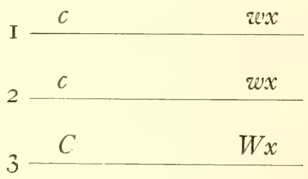


Diagram 1

Now if, at any division of an endosperm nucleus, the two halves (*a* and *b*) resulting from a longitudinal split of the chromosome carrying *C* and *Wx* (chromosome 3 of diagram 1) should fail to separate (non-disjunction) and should go together to one pole (*A*), the resulting daughter nuclei (*A* and *B*) would be as shown in diagram 2.

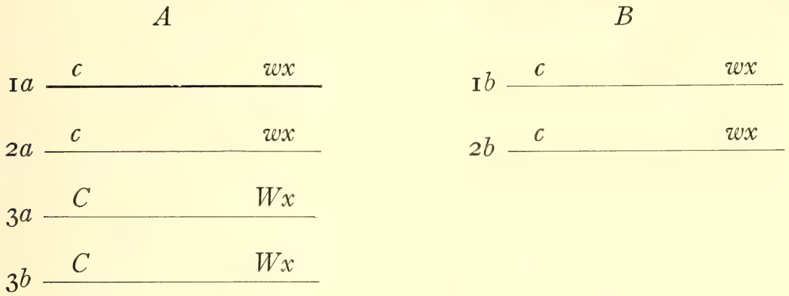


Diagram 2

Obviously nucleus "*B*" and all its descendants would lack both *C* and *Wx* so that the resulting aleurone would be colorless and its underlying endosperm waxy, while the aleurone and endosperm cells resulting from the further division of nucleus "*A*" would be colored and corneous. The same results would follow if chromosome 3 failed to divide, going entire to one pole, or if after equational division one of the halves were left behind, failing to reach either pole.

If, on the other hand, the colorless part of an aberrant seed be due to a somatic mutation of *C* to *c*, there is no reason to suppose that the same mutation would change *Wx* to *wx*. From what is known of the origin of factor ("point") mutations, there is little if any more warrant for the assumption that a single mutation will ordinarily involve simultaneously two loci of one chromosome than that it will affect loci of non-homologous chromosomes. If, therefore, in the case under consideration, the colorless part of an aberrant seed be due to a somatic mutation, the endosperm underlying it should be corneous, *c c c wx wx Wx*, like that underlying the colored part of the aleurone, *c c C wx wx Wx*.

It now remains to examine the evidence derived from crosses of colorless waxy individuals, *c Wx*, with pollen of colored corneous ones, *C Wx*, and to determine whether the colorless parts of aberrant seeds resulting from such crosses are underlaid with waxy or with corneous endosperm. The data available from the writer's cultures are presented in table 1. Of the 65 aberrant seeds there recorded, the part with colorless aleurone was underlaid by waxy endosperm in 55 cases, by corneous endosperm in 3 cases, and in the remaining 7 cases the endosperm texture could not be determined either because of the extremely small size of the colorless spots or because of the immaturity of the seeds.

The aberrant parts of these seeds varied in area from not much more than a square millimeter to about two thirds of the entire surface of the seed, 32 of the 65 seeds having one sixth or more and only 4 having more than one half of the surface colorless. Eight of the 65 seeds had numerous colorless spots of varied sizes but mostly small, and all the others had only a single spot each. The line of demarcation between the colored and colorless parts was invariably sharp but usually somewhat irregular. The correspondence in outline between the colorless aleurone and the underlying waxy endosperm was strikingly exact irrespective of the number of spots per seed or of their irregularity (fig. 1; *I*, *J*, *K*, *L*). The waxy endosperm was found to extend to varying depths, the smaller spots often being more shallow than the larger ones (fig. 1; *K*, *L*). Moreover, the larger waxy parts often exhibited a somewhat irregular outline in cross-section (fig. 1, *K*). It is perhaps possible that the three seeds noted as having corneous endosperm under the colorless aleurone had in reality a very shallow layer of waxy endosperm, but this is not likely since in neither case did the colorless spot include less than about one fourth of the entire area of the seed.

The writer has examined three aberrant seeds involving *C c* and *Wx wx* from cultures other than his own and in each case the colorless aleurone was directly over waxy endosperm. The seed described by G. N. Collins (1913), from F_2 of a cross of white waxy with pollen of colored non-waxy types, in which the colorless aleurone was underlaid by waxy and the colored part by corneous endosperm, involved, it is now almost certain, *C c* with *Wx wx*. Collins's published F_2 records leave no doubt that he was dealing with a case of linkage between waxy endosperm and some aleurone factor. The cross certainly did not involve the aleurone factor pair *I i*, for the colorless condition was recessive. Aleurone-color factors *A a* (Bregger, 1918) and *R r* (Kempton, 1919) are now known to be inherited independently of *Wx wx*, so that *C c* is the only known factor pair that could have been involved. Since, however, Collins's case appeared in F_2 and since there is about 25 percent of crossing-over between *C c* and *Wx wx*, there is no certainty that both *C* and *Wx* were in one chromosome and *c* and *wx* in another.

The evidence derived from crosses involving the linked genes *C-Wx* and *c-wx* points conclusively—in so far as genetic evidence can be regarded as at all conclusive with respect to cytological behavior—to some aberrant chromosome distribution, perhaps non-disjunction, as the cause of most cases of aberrant endosperm development; but the three instances noted above of corneous endosperm underlying colorless spots of aleurone suggest, though they do not prove, that very rarely somatic mutation may be responsible.

Evidence from other linked factors in addition to *C c* and *Wx wx* would be of great value as tending to confirm or contradict the conclusion here drawn. A number of such linkages are now known. In addition to the linkage of *I i* with *Wx wx*, inferred, as noted earlier in this paper, from Hutchison's data, Hutchison has found both *I i* and *C c* to be closely linked with shrunken endosperm, *Sh sh*, and Dr. E. G. Anderson (unpublished data) has noted linkage between a factor pair for blotched aleurone, *Bh bh*, and the pair *Y y* for yellow endosperm.

The writer has not been able as yet to obtain from his own cultures aberrant seeds involving any of these linkages, but in Professor Hutchison's material a single aberrant seed involving *C c* and *Sh sh* has been observed. A colorless- and shrunken-seeded plant, *c sh*, pollinated by a plant heterozygous for these factors, *C c Sh sh*, produced the aberrant seed. It was

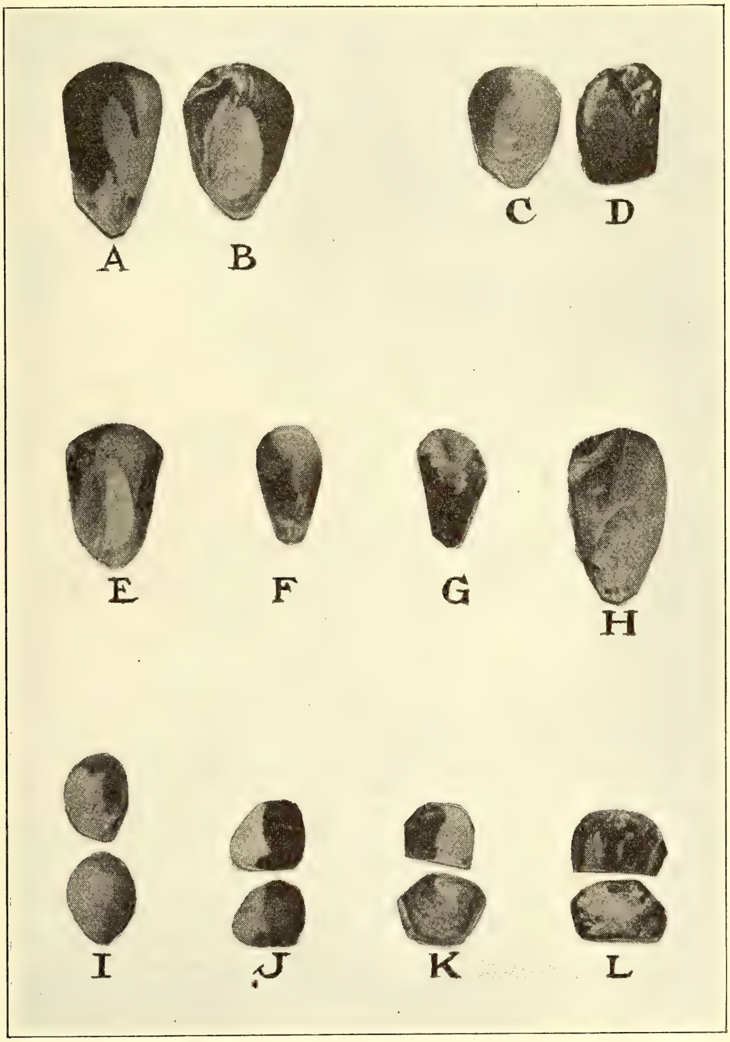


FIG. 1. Aberrant endosperm in maize seeds. Factors involved: A and B, *R r Su su;* C and D, *C c Su su;* E, *R r Wx wx;* F, *A a Wx wx;* G, *C c Sh sh;* H, *Su su;* I to L, *C c Wx wx*. In I and J the upper figures show untreated seeds with purple-colorless aleurone, and the lower figures show the same seeds after the pericarp and aleurone layer have been removed, the corneous endosperm appearing dark and the waxy endosperm light. In K and L, the upper figures represent parts of untreated seeds and the lower figures corresponding cross sections of the same seeds.

colored and non-shrunken, *C Sh*, except for a single large spot that was both colorless and shrunken (fig. 1, G). The sperm in this case must have carried both *C* and *Sh*, for otherwise the seed would have been colorless and shrunken throughout. The evidence, therefore, so far as this one seed is concerned, is definitely in favor of the hypothesis of aberrant chromosome behavior and opposed to that of somatic mutation.

Both *I i* and *Wx wx* are without doubt concerned in the case of a single aberrant seed reported by G. N. Collins (1913). A colored waxy type pollinated by a colorless corneous one resulted in colorless corneous seeds. The dominance of the colorless condition establishes, so far as is now known, the presence of *I* in the colorless pollen parent. A single seed of this cross, though colorless and corneous in the main, had a small spot of colored aleurone which overlaid exactly a spot of waxy endosperm. It seems evident that in this one instance in which an aberrant seed involved the linked factors *I* and *Wx*, just as in the single case in which *C* and *Sh* were involved and in the great majority of the cases—55 out of 58—in which the linked factors *C* and *Wx* were concerned, aberrant endosperm development is ordinarily due to some unusual chromosome behavior possibly of the nature of non-disjunction.

One unfamiliar with some of the results previously published will not have failed to observe by this time that either one of Webber's (1900) well-known hypotheses might account for the results presented above quite as well as the hypothesis of non-disjunction. Webber, it will be recalled, suggested as possible explanations of aberrant endosperm development (1) that the second sperm nucleus on the one hand and the fused polar nuclei on the other may occasionally develop independently, each giving rise to a part of the endosperm, or (2) that the second sperm nucleus may sometimes unite with one polar nucleus, leaving the other polar nucleus to develop independently. If either of these things should happen, it is obvious that, in cases of aberrant endosperm where *C* and *Wx* come from the male and *c* and *wx* from the female parent of a cross, the colored parts must be corneous and the colorless ones waxy. The second sperm nucleus, carrying *C* and *Wx*, whether it divide independently or unite with one polar nucleus, would give rise to colored corneous endosperm, and the endosperm developed either from one polar nucleus alone or from a fusion of the two, both carrying *c* and *wx*, would produce colorless waxy endosperm.

But it was shown by East (1913) that Webber's first hypothesis, independent development of the sperm nucleus and of the fused polar nuclei, was untenable. Crosses were made between two types of maize, both with colorless aleurone but one having factor *C* and the other having factor *R*, both of which are essential to aleurone-color development. Among the numerous colored seeds resulting from these crosses, six were colored on one side and colorless on the other. It is obvious that these aberrant seeds could not have arisen in accordance with Webber's first hypothesis, for, since the second sperm nucleus carried *C* and not *R* and the polar nuclei *R* and not *C*, if the two elements divided without undergoing fusion, *C* and *R* could never have been brought together and no aleurone color could have developed in any part of the seeds.

TABLE 1. *Aberrant seeds of maize from crosses of colorless waxy, c wx, by colored corneous, C Wx*

Pedigree No. of ♀ Parent	Approximate Number of Normal Seeds	Number of Aberrant Seeds. Endosperm under Colorless Parts		
		Waxy	Corneous	Undetermined
9062– 2	460	2		
3	600	3		
3	500	1		
6	280	1	1	
9064– 2	10	1		
9491– 1	560	1		1
2	250	1		
10168– 5	480	1		
10169– 3	430	1		
10170– 1	400	2		
2	300	2		
2	460	1		
4	390	4		1
5	310			1
7	70	1		
10171– 1	460	2		1
4	450	1		
6	650	1		
7	620	2	1	
8	340	2		
10	430	2		
11	290	1		
11	540	1		
13	160	1		
14	560	3		
16	530	1		
17	270	1		
18	630	3		
18	470	2		
19	190	2		
23	240	1		
24	350	2		
25	140	1		
27	470	2		
29	430			1
10182– 1	150			2
5	240	1	1	
11	120	1		
29 ears	9,580	0	0	0
Total 67 ears	23,810	55	3	7

In a similar way the writer (Emerson, 1915) was able to show that Webber's second hypothesis is incorrect. Two colorless types, each having only one of the two complementary aleurone-color factors *C* and *R*, were crossed as in the experiments of East. In addition, the type used as the female parent was sugary and the one used as the male parent was starchy.

Among the resulting seeds, which were colored and starchy as expected, occurred two with aberrant endosperm, one of which was part colored and part colorless but starchy throughout and the other one part starchy and part sugary but colored throughout. As in the case reported by East, no color could have developed if the second sperm nucleus had not fused with a polar nucleus. Furthermore, if the second sperm nucleus had united with one polar nucleus (Webber's second hypothesis), the part of the endosperm so formed must have been both colored and starchy while the remaining part of the endosperm formed from the other polar nucleus alone must have been both colorless and sugary. The observed facts, namely, that the starchy-sugary seed was colored throughout and that the colored-colorless one was starchy throughout, indicated clearly that normal fusion of the second sperm nucleus with the polar nuclei had taken place.

It remains to forestall the justifiable criticism that one or other of Webber's hypotheses might still account for most examples of anomalous endosperm, the two seeds noted above being minor exceptions, just as either of these hypotheses might well be used to explain the 55 aberrant seeds with colorless spots waxy recorded in table 1, the 3 seeds with colorless spots corneous likewise being exceptions. In the writer's 1918 paper (tables 8 and 10) were recorded 33 examples of anomalous seeds with part colored and part colorless aleurone from crosses between types with wholly colorless aleurone but carrying complementary aleurone-color factors. These, added to the six cases reported by East (1913), a total of 39, are believed to suffice as a demonstration that division of the second sperm nucleus independently of the fused polar nuclei is quite untenable. Moreover, two of the 33 anomalous seeds afforded definite evidence against the hypothesis that one polar nucleus might fuse with the second sperm nucleus and the other polar nucleus divide independently, making a total of four such instances. These two seeds resulted from a cross of a type with colorless sugary endosperm and colorless aleurone carrying *R* with pollen of a type with yellow starchy endosperm and colorless aleurone carrying *C*. The aberrant seeds were starchy and yellow throughout but their aleurone was about half colorless and half purple.

Since the publication of the writer's 1918 paper, a sufficient mass of evidence has been obtained to remove, it is thought, any possibility of explaining anomalous endosperm development on the basis of a failure of normal fusion of the second sperm nucleus and the polar nuclei. This additional evidence is presented in tables 2 and 3.

In table 2 is recorded all the available material in which corneous and waxy endosperm, *Wx wx*, are involved together with the aleurone-factor pairs *A a*, *R r*, and *Pr pr*. The male parents of all the crosses here recorded had homozygous corneous endosperm and homozygous purple aleurone, *A C R Pr Wx*, while the female parents of all had waxy endosperm, *wx*. In addition, the female parents of all crosses recorded in groups 1 and 2 of the table had colorless aleurone, *a C R* for group 1 and *A C r* for group 2, and those of the crosses shown in group 3 had red aleurone, *A C R pr*. In all, 38 aberrant seeds are reported, 12 of which involved *A a Wx wx* (fig. 1, F), 6 *R r Wx wx* (fig. 1, E), and 20 *Pr pr Wx wx*. In all these cases, the recessive aleurone color of the female parent, namely, colorless in groups 1 and 2 and red in group 3, occurred in the aberrant part of the seed, and the dominant color, purple, of the male parent occurred in the normal part. But in every instance the endosperm was corneous throughout like that of

TABLE 2. *Aberrant seeds of maize from crosses of colorless waxy, a wx and r wx, by colored corneous, A R Wx, and of red waxy, pr wx, by purple corneous, Pr Wx*

Group	Genes Concerned	Pedigree No. of ♀ Parent	Approximate Number of Normal Seeds	Number of Aberrant Seeds, all Corneous
				Colored and colorless
1..........	*a wx* × *A Wx*....	9061– 3	300	2
		5	500	1
		10183– 2	500	2
		8	350	3
		11	520	1
		14	280	2
		15	240	1
		12 ears	4,610	0
Total.......		19 ears	7,100	12
2..........	*r wx* × *R Wx*....	10187– 5	290	1
		6	440	1
		8	240	2
		9	190	2
		2 ears	560	0
Total.......		6 ears	1,720	6
				Purple and red
3..........	*pr wx* × *Pr Wx* ..	9062– 2	460	1
		5	470	1
		10171– 4	450	1
		6	650	1
		9	590	1
		14	560	1
		15	540	2
		18	630	2
		18	470	1
		23	280	1
		27	470	1
		10182– 1	390	1
		2	300	1
		5	480	2
		9	390	1
		10183– 7	280	1
		10187– 6	440	1
		8	240	1
		6 ears	2,220	0
Total.......		24 ears	10,310	20

the male parent. No case of aberrant corneous-waxy endosperm was observed, but there is no satisfactory evidence that none occurred among the wholly colored seeds, where waxy spots would be easily overlooked.

Similarly, in table 3 are recorded all available cases in which starchy and sugary endosperm, *Su su*, are involved together with the aleurone-factor pairs *C c*, *R r*, and *Pr pr*. The male parents of all these crosses had homozygous starchy endosperm and homozygous purple aleurone, *A C R*

TABLE 3. *Aberrant seeds of maize from crosses of colorless sugary, c su and r su, by colored starchy, C R Su, and of red sugary, pr su, by purple starchy, Pr Su*

Group	Genes Concerned	Pedigree No. of ♀ Parent	Approximate Number of Normal Seeds	Number of Aberrant Seeds: All Starchy, Colored and Colorless	Number of Aberrant Seeds: All Colored, Starchy and Sugary
1........	*c su* × *C Su*...	9063– 1	260	1	
		3	240	1	
		4	320	2	
		5	179	1	1
		7	510	1	
		10178– 2	320	2	
		3	290	1	
		4	410	2	1
		5	290		1
		6	370	2	
		6	220	1	
		10179– 1	420	1	
		2	360	1	
		4	250		1
		5	380		1
		6	430	3	
		6	440	1	
		5 ears	1,750	0	0
Total....		22 ears	7,430	20	5
2........	*r su* × *R Su* ..	8572– 1	190	2	
		10185– 1	420	2	2
		2	320	1	
		4	400	2	1
		5	390		1
		6	380	1	
		7	290	2	
		7	440	1	
		10	360	2	2
		7 ears	2,720	0	0
Total....		16 ears	5,910	13	6
				All Starchy, Purple and Red	All Purple, Starchy and Sugary
3........	*pr su* × *Pr Su*	10180– 1	360		1
		5	260	1	
		7	220		1
		5 ears	710	0	0
Total....		8 ears	1,550	1	2

Pr Su, while the female parents of all had sugary endosperm, *su*. In addition, the female parents of all crosses shown in groups 1 and 2 of the table had colorless aleurone, *A c R* for group 1 and *A C r* for group 2, and those of the crosses presented in group 3 had red aleurone, *A C R pr*. In all, 47 aberrant seeds are recorded. Of these, 34 had aberrant aleurone color, 20 involving *C c Su su* (fig. 1, C), 13 *R r Su su* (fig. 1, A), and 1 *Pr pr Su su*; and 13 had aberrant endosperm texture, 5 involving *C c Su su* (fig. 1, D), 6 *R r Su su* (fig. 1, B), and 2 *Pr pr Su su*. Every one of the 34 seeds that had aberrant aleurone (colored-colorless) were starchy throughout, and all of the 13 with aberrant endosperm (starchy-sugary) had colored aleurone throughout.

In short, there have been observed (tables 2 and 3) a total of 85 aberrant seeds involving *C c* with *Su su*, *A a* with *Wx wx*, and *R r* and *Pr pr* with both *Su su* and *Wx wx*. In every case in which aleurone color was concerned, 72 in all, the aberrant spot showed the recessive color of the female parent but was invariably underlaid with the dominant corneous or starchy endosperm of the male parent; and in all of the 13 cases in which endosperm composition was concerned the aberrant spot exhibited the recessive sugary condition of the female parent but was invariably overlaid by the dominant aleurone color of the male parent. It is obvious, therefore, that for none of the 85 seeds could the aberrant spots, though they displayed in every case one or other (never both) of the two recessive maternal characters whose genes were carried in the polar nuclei, have been produced by the independent division either of one polar nucleus alone or of the two after fusion. To explain these cases on the basis of independent division of one or both polar nuclei would require the unwarranted additional assumption that in some cases—aberrant sugary spots—the polar nucleus or nuclei alone give rise to a part of the underlying endosperm but to none of the aleurone layer, while in other cases—aberrant aleurone-color spots—they give rise to a part of the aleurone but to none of the underlying endosperm. Moreover, if such behavior of independently dividing polar nuclei were so common in cases involving the endosperm factors *Wx wx* with the aleurone factors *A a*, *R r*, and *Pr pr* (table 2), and the endosperm factors *Su su* with the aleurone factors *C c*, *R r*, and *Pr pr* (table 3), why should not the same behavior of the polar nuclei be found where there is involved *Wx wx* with *C c* (table 1) or with *I i* (Collins, 1913) or *C c* with *Sh sh* (Hutchison's data)? But the facts are that in the great majority of aberrant seeds involving *C c* and *Wx wx* (55 to 3) where the aleurone layer is colorless (maternal), the underlying endosperm is waxy (also maternal), and the correspondence in outline between colorless aleurone and waxy endosperm is strikingly exact. Certainly no single hypothesis that assumes independent development of either one or both of the polar nuclei can be made to fit all the data now available.

That the somatic-mutation hypothesis suggested by the writer (1915) does not agree with the great majority of the observed facts when *C c* and

Wx wx are concerned, just as the hypotheses of independent division of polar nuclei suggested by Webber (1900) do not fit the available facts where other than these aleurone and endosperm factors are concerned, was shown earlier in this paper. The hypothesis of vegetative segregation (East and Hayes, 1911) is not sufficiently specific with respect to the mechanism of such supposed segregation to make it possible to apply crucial tests. Moreover, several cases of somatic variations often referred to as cases of vegetative segregation are quite as likely due to somatic mutation. There remains only the hypothesis of aberrant chromosome behavior (non-disjunction?) which is in accord with practically all the reported cases of aberrant endosperm development. It was shown earlier how that hypothesis fits the cases involving the linked genes *C c* (or *I i*) with *Wx wx*. That this hypothesis is not in disagreement with the cases where other endosperm and aleurone factors are concerned follows from the fact—determined by ordinary breeding tests—that these other factors are not genetically linked and that, therefore, they presumably have their loci in non-homologous chromosomes. Evidence of non-linkage for *A a* with *Wx wx* was presented by Bregger (1918), for *R r* with *Wx wx* by Kempton (1919), and for *Su su* with *C c*, *R r*, and *Pr pr* by Eyster (1921); and there is indirect evidence for *Pr pr* and *Wx wx* in Hutchison's data which show *Wx wx* to be linked with *Sh sh* and the latter to be independent of *Pr pr*. If none of these combinations of genes lies in the same chromosome, it is obvious that a non-disjunction of one chromosome could not affect more than one member of the combination, just as it is that both members of any combination lying in one chromosome must be affected by a single non-disjunction of that chromosome.

While the writer feels that the genetic evidence in favor of the hypothesis of non-disjunction, or at least of some aberrant chromosome behavior giving a similar result, as the cause of most cases of the kind of aberrant endosperm here discussed is as convincing as such evidence can well be, it is realized that direct proof must come, if at all, from cytological studies. Whether it will ever be possible to detect non-disjunction cytologically in the endosperm of maize, granting that it occurs, cannot be said. The small size of maize chromosomes and their large number, 30 in the triploid nucleus, increase the difficulty of the undertaking. Moreover, the rarity of the phenomenon lessens the chance of a successful outcome. On this latter point, however, there is this to be said: non-disjunction is doubtless a much more common occurrence than are its visible manifestations. There is no reason to suppose, for instance, that, in material of the genotype *c c C wx wx Wx*, such as that recorded in table 1, the chromosome carrying *C* and *Wx* is more often concerned in non-disjunction than are the other two homologous chromosomes each carrying *c* and *wx*. But a non-disjunction involving either of the latter could result in no visible change in either the color of the aleurone or the texture of the endosperm. It may well be assumed, therefore, that non-disjunction within this one group of chromosomes occurs three times as frequently as it is visibly manifested in such material.

Moreover, there is no satisfactory evidence that one group of homologous chromosomes is involved more frequently than any one of the other nine groups. It may be supposed, therefore, that non-disjunction occurs on the average 30 times for every aberrant seed observed in material heterozygous for a single factor pair. Certainly non-disjunction—if such be the cause of aberrant endosperm—is not limited to the *C-I-Sh-Wx* chromosome. Aberrant endosperm has been observed to involve the additional aleurone and endosperm factors *A*, *R*, *Pr*, *Y*, and *Su*, all of which are inherited independently of the *C-I-Sh-Wx* group, and all of which, except possibly *R* and *Pr*, are inherited independently of one another. Since, therefore, aberrant endosperm has been observed to involve not less than five and perhaps six linkage groups, aberrant endosperm behavior is assumed to have occurred in at least the same number of groups of homologous chromosomes and there is no reason to suppose that it is not common to all ten groups.

From tables 1–3 of this paper, it is seen that 150 aberrant seeds were observed with an approximate total of 57,830 normal seeds. Of these 150 seeds, 13 involved sugary endosperm, a character that might easily be overlooked except when the aberrant spot is large. Since, moreover, the material involving sugary endosperm had to do also with an aleurone-color factor, the 13 seeds must be omitted if we are to deal with a single factor or linked-factor group, in other words, with a single chromosome, at a time. The observed ratio, when only one factor is involved, is 57830 : 137, or approximately one aberrant case in every 423 seeds. If now it be assumed that non-disjunction occurs thirty times as frequently as aberrant seeds in such material, non-disjunction should occur on the average once in about 14 seeds. In more than half of the aberrant seeds reported in this paper (77 out of 150) the aberrant part included approximately one sixth or more of the surface area of the seed and in about one twelfth of them it included more than one half of the seed. Consequently, non-disjunction must occur, if at all, fairly early in endosperm development in a considerable percentage of cases. It would seem worth while, therefore, for cytologists to search for it at least in the early divisions of the endosperm nucleus.

An observation noted earlier in this paper suggests that irregularities besides non-disjunction may occur in endosperm development. It was noted that 8 out of 65 aberrant seeds involving *C c* and *Wx wx* were mottled, exhibiting numerous small spots of colorless aleurone instead of a single spot. One of these mottled seeds was so immature that the endosperm texture could not be determined, but in the other seven the colorless spots were underlaid by waxy endosperm (fig. 1, L), this association indicating definitely aberrant chromosome behavior. It does not seem likely that non-disjunction would occur repeatedly in the development of a single seed, but if it does not there must have been very irregular migration of endosperm nuclei after non-disjunction occurred. In material involving *C c Su su* and *A a Wx wx*, 6 of the 22 seeds showing aberrant aleurone color were mottled. Practically all of the normal seeds in material where *R r* is involved were mottled, and the aberrant seeds showed mottling in the colored part (fig. 1, A and E), but mottling is known to occur commonly in aleurone heterozygous for *R* when *R* enters with the sperm and *r r* with the polar nuclei and, whatever its ultimate cause, it is not to be confused with what is here termed aberrant endosperm.

Summary

It has been shown that, when aberrant seeds occur in crosses in which recessive aleurone and endosperm characters are contributed by the female parent and the corresponding dominant characters by the male parent, spots of the recessive (maternal) aleurone color are in the great majority of cases underlaid by the recessive (maternal) type of endosperm if the genes for these aleurone and endosperm characters are genetically linked, as shown by breeding tests, while similar recessive aleurone-color spots are always, so far as observed, underlaid by the dominant (paternal) type of endosperm and recessive endosperm parts are overlaid by the dominant aleurone color if the aleurone-color and endosperm-composition genes are not linked.

These facts are held to support the hypothesis of occasional aberrant chromosome behavior—possibly non-disjunction—and are incompatible with the earlier hypotheses involving failure of normal fusion of the second sperm nucleus with the polar nuclei, and also make untenable, except in rare cases, the hypothesis of somatic mutation.

LITERATURE CITED

Bregger, T. Linkage in maize: the *C* aleurone factor and waxy endosperm. Amer. Nat. **52**: 57–61. 1918.

Collins, G. N. Mosaic coherence of characters in seeds of maize. U. S. Dept. Agr., Bur. Plant Ind., Circ. **132**: 19–21. 1913.

Collins, J. L. Chimeras in corn hybrids. Jour. Hered. **10**: 3–10. 1919.

East, E. M. Xenia and the endosperm of angiosperms. Bot. Gaz. **56**: 217–224. 1913.

——**, and Hayes, H. K.** Inheritance in maize. Conn. Agr. Exp. Sta. Bull. **167**: 1–141. 1911.

Emerson, R. A. Anomalous endosperm development and the problem of bud sports. Zeitschr. induk. Abstamm. Vererb. **14**: 241–259. 1915.

——. A fifth pair of factors, *A a*, for aleurone color in maize, and its relation to the *C c* and *R r* pairs. Cornell Univ. Agr. Exp. Sta. Memoir **16**: 225–289. 1918.

Eyster, W. H. The linkage relations between the factors for tunicate ear and starchy-sugary endosperm in maize. Genetics **6**: 209–240. 1921.

Hutchison, C. B. Heritable characters of maize VII. Shrunken endosperm. Jour. Hered. **12**: 76–83. 1921.

Kempton, J. H. Inheritance of waxy endosperm in maize. U. S. Dept. Agr. Bull. **754**: 1–99. 1919.

Webber, H. J. Xenia, or the immediate effect of pollen in maize. U. S. Dept. Agr., Div. Veg. Phys. Path. Bull. **22**: 1–44. 1900.

Announcing the publication of

An Introduction to CYTOLOGY

By LESTER W. SHARP

Assistant Professor of Botany, Cornell University

452 pages, 6 x 9, 159 illustrations, $4.00

McGraw-Hill Agricultural and Biological Publications

A book for students of the biological sciences who desire a means of becoming more readily acquainted with the literature and problems of cytology. Throughout the book attention is focussed upon the protoplast; the cell wall is given only brief consideration, since it plays a relatively minor role in the processes of particular interest to the cytologist at the present time. Because of their fundamental importance in connection with the problems confronting the geneticist, the phenomena of nuclear division, chromosome reduction, and fertilization are described with considerable fullness, and their relation to the problems of heredity is taken up in five special chapters.

Professor Charles J. Chamberlain, of the University of Chicago, says of this book:

"Such a book has long been needed by botanists and also by specialists in genetics, plant breeding and pathology. Professor Sharp has an international reputation as a cytologist. There is no botanist in the world better qualified to write such a book."

Professor R. A. Emerson, of Cornell, says:

"It is fortunate that we have in America a cytologist who is thoroughly familiar not only with both plant and animal cytology but also with the bearing of cytology on the problems of heredity. I regard the chapters on the interrelations of cytology and genetics as without exception the best presentation of the subject I have seen."

Other New Volumes in Our Series of
McGraw-Hill Agricultural and Biological Publications
(Dr. Charles V. Piper, Consulting Editor)

Hayes and Garber—BREEDING CROP PLANTS	**$3.50**
Fernald—APPLIED ENTOMOLOGY	**3.50**
Thatcher—CHEMISTRY OF PLANT LIFE	**3.00**

Send for copies of these books on approval

McGRAW-HILL BOOK CO., Inc.
Penn Terminal Building
370 Seventh Avenue NEW YORK

The Little Things That Count Big In Owning Your Own Greenhouse

Ever gone into a hat store, knowing exactly the kind and shaped hat you want; and then have some smart Alec of a salesman try to sell you the kind HE likes?

Makes a fellow madder than a wet hen, doesn't it?

Suppose, however, that same salesman had told you something about some of his hats that meant better service to you—more satisfaction for your money—you then sort of feel towards that salesman as you would an old friend.

Salesman number two is our sort when it comes to selling a greenhouse.

We do sell greenhouses, but we first sell satisfaction.

Catalog for the asking.

Hitchings and Company

Home Office and Factory: ELIZABETH, N. J.

NEW YORK
101 Park Ave.

BOSTON-9
294 Washington St.

Vol. VIII. NOVEMBER, 1921 No. 9

AMERICAN
JOURNAL OF BOTANY

OFFICIAL PUBLICATION OF THE
BOTANICAL SOCIETY OF AMERICA

Smithsonian Institution
DEC 21 1921
National Museum

CONTENTS

The interrelationship of the number of the two types of vascular bundles in the transition zone of the axis of Phaseolus vulgaris.
J. Arthur Harris, Edmund W. Sinnott, John Y. Pennypacker and G. B. Durham 425

Area of vein-islets in leaves of certain plants as an age determinant
M. R. Ensign 433

Unusual rusts on Nyssa and Urticastrum . . . . E. B. Mains 442

Miscellaneous studies on the crown rust of oats . . . . . . G. R. Hoerner 452

Comparative studies on respiration XVIII. Respiration and antagonism in Elodea. . . . . C. J. Lyon 458

The effect upon permeability of (I) the same substance as cation and anion, and (II) changing the valency of the same ion . . . . Oran L. Raber 464

PUBLISHED
IN COOPERATION WITH THE BOTANICAL SOCIETY OF AMERICA
BY THE
BROOKLYN BOTANIC GARDEN
At 41 North Queen Street, Lancaster, Pa.

Entered as second-class matter February 21, 1914, at the post office at Lancaster, Pennsylvania, under the act of March 3, 1879

AMERICAN JOURNAL OF BOTANY

Devoted to All Branches of Botanical Science

Established 1914

EDITED BY A COMMITTEE OF THE
BOTANICAL SOCIETY OF AMERICA

EDITORIAL COMMITTEE

C. E. Allen, Editor-in-Chief,
University of Wisconsin

William Crocker,
The Thompson Institute For Plant Research, Inc.

L. R. Jones,
University of Wisconsin

C. Stuart Gager, Business Manager
Brooklyn Botanic Garden

Arthur H. Graves
Brooklyn Botanic Garden

R. A. Harper,
Columbia University

Jacob R. Schramm,
Cornell University

C. L. Shear, *Bureau of Plant Industry*
(Representing American Phytopathological Society)

The Journal is published monthly, except during August and September. Subscription price, $6.00 a year. Single copies 75 cents. Back numbers, 75 cents each; $7.00 a volume. Postage will be charged to all foreign countries, except Mexico, Cuba, Porto Rico, Panama Canal Zone, Republic of Panama, Hawaii, Philippine Islands, Guam, Samoan Islands, and Shanghai. Postage to Canada, 20 cents a volume on annual subscriptions; to all other countries in the Postal Union, 40 cents a volume on annual subscriptions.

The pages of the Journal are open to members of the Botanical Society of America, or to candidates approved for membership.

Manuscript offered for publication should be typewritten, and should in all cases be submitted to the Editor-in-Chief.

Papers are limited to 20 pages in length, exclusive of plates, except that additional pages may be arranged for at cost rate to authors (approximately $3.00 a page).

Proofs should be corrected immediately on receipt, and returned to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y.

Illustrations, other than zinc etchings, and tabular matter should, in general, be restricted to approximately ten percent of the number of pages of text. Authors may be charged at cost rates for illustrations and tabular matter in excess of that amount (approximately $6.40 a page for tabular matter; 15 cents per square inch for half-tones).

Separates should be ordered when proof is returned. Fifty copies without cover will be supplied free; cover and additional copies at cost.

Remittances should be made payable to American Journal of Botany. Ten cents must be added to all checks not drawn on New York City banks.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

Correspondence concerning editorial matters should be addressed to Prof. C. E. Allen, University of Wisconsin, Madison, Wisconsin.

Business correspondence, including notice of change of address, and directions concerning reprints, should be addressed to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y., or 41 North Queen Street, Lancaster, Pa.

AMERICAN
JOURNAL OF BOTANY

VOL. VIII NOVEMBER, 1921 NO. 9

THE INTERRELATIONSHIP OF THE NUMBER OF THE TWO TYPES OF VASCULAR BUNDLES IN THE TRANSITION ZONE OF THE AXIS OF PHASEOLUS VULGARIS

J. ARTHUR HARRIS, EDMUND W. SINNOTT, JOHN Y. PENNYPACKER, AND G. B. DURHAM

(Received for publication January 17, 1921)

INTRODUCTORY

In papers[1] on the anatomy of dimerous[2] and trimerous and of hemitrimerous seedlings we have shown that *Phaseolus vulgaris* is characterized by a structure of the vascular system at the base of the hypocotyl which is rather infrequent in seedling anatomy in general. This is the presence of a variable number of accessory bundles which usually lack protoxylem elements. These are the "Zwischenstränge" of Dodel. We have elsewhere called them intercalary bundles. They may make their appearance in the upper part of the root or in the lower region of the hypocotyl, some rising blindly below and others originating by division of a primary double bundle. These intercalary strands may be distinguished from the other bundles with perfect certainty because of their position and of the absence within them of any protoxylem elements.

In another place[3] we have dealt with the correlations between the number of bundles at different levels in the seedling, that is, the relationship between the vascular system at the base of the hypocotyl and that in the central region of the hypocotyl and epicotyl, and between the bundle system in the hypocotyl and that in the epicotyl. Our present problem is to consider the interrelationships between the two types of bundles present in the hypocotyl just above the region of transition from root to stem structures, and between each of these types and the total number of bundles in this zone.

---

[1] Harris, J. Arthur, Sinnott, E. W., Pennypacker, J. Y., and Durham, G. B. The vascular anatomy of dimerous and trimerous seedlings of *Phaseolus vulgaris.* Amer. Jour. Bot. **8**: 63–102. 1921. The vascular anatomy of hemitrimerous seedlings of *Phaseolus vulgaris.* Amer. Jour. Bot. **8**: 375–381. 1921.

[2] Dimerous seedlings have 2 cotyledons and 2 primordial leaves; trimerous seedlings have 3 cotyledons and 3 primordial leaves; and hemitrimerous seedlings have 3 cotyledons and 2 primary leaves.

[3] Harris, J. Arthur, Sinnott, E. W., Pennypacker, J. Y., and Durham, G. B. Correlations between anatomical characters in the seedling of *Phaseolus vulgaris.* Amer. Jour. Bot. **8**: 339–365. 1921.

[The **Journal** for October (8: 375–424) was issued November 14, 1921].

Lack of space precludes the publication of the 30 individual correlation tables upon which the coefficients discussed in this section are based. These may, however, be easily formed from the schedules showing the formula for the basal bundles in other papers of this series.[4]

TABLE I. *Correlation between Number of Primary Double Bundles and Number of Intercalary Bundles at Base of Hypocotyl*

Line	Trimerous			Dimerous			Difference	$\frac{\text{Diff.}}{E_{\text{diff.}}}$
	N	r	$\frac{r}{E_r}$	N	r	$\frac{r}{E_r}$		
75	142	−.5004±.0424	11.8	142	−.1177±.0558	2.11	−.3827±.0700	5.46
93	155	−.6155±.0337	18.3	155	−.1449±.0530	2.73	−.4706±.0624	7.54
98	183	−.6515±.0286	22.8	183	+.0643±.0496	1.30	−.7158±.0574	12.4
139	106	−.5053±.0488	10.4	305	+.1364±.0379	3.60	−.6417±.0618	6.0
143	221	−.3184±.0408	7.8	420	+.0338±.0329	1.03	−.3522±.0530	5.4

ANALYSIS OF DATA

1. *Relationship between Number of Primary Double Bundles and Number of Intercalary Bundles.* We shall first consider the relationship between the number of primary double bundles and the number of intercalary bundles at the base of the hypocotyl in dimerous and trimerous plants.

The correlation coefficients for the five lines appear in table 1. For the trimerous plants of all five lines the correlations are negative in sign, *i.e.*, the number of intercalary bundles is greater in plants which have a smaller number of primary double bundles, and *vice versa*. For dimerous plants three of the five lines show a slightly negative coefficient, but two show a low positive correlation. The constants indicate that the correlations for the trimerous plants are much higher numerically than those for the dimerous plants. Those for the trimerous are of the order −.3 to −.6 while those for dimerous plants are sensibly zero, averaging + .005. The correlations for the trimerous plants are in all cases several times as large as their probable errors, while those for the dimerous plants could hardly be regarded as statistically significant if only one of the lines were available. The differences, taken with regard to sign, between the correlations for the dimerous and trimerous plants are in each case significant in comparison with their probable errors.

Expressing these results in terms of regression we have the following equations:

---

[4] The entries to be selected from the published tables can be determined from the values of N. In lines in which true siblings were available (75, 93, and 98) only siblings have been used, even though additional sections of one or the other type were available. In the two lines in which random samples of seed were used for the production of the dimerous and trimerous seedlings, the largest possible number of individuals available in the tables of the papers cited was employed for the constants here discussed.

	Dimerous	Trimerous
Line 75:	$P = 4.255 - 0.058\ I$	$P = 6.059 - 0.318\ I$
	$I = 1.641 - 0.239\ P$	$I = 4.968 - 0.789\ P$
Line 93:	$P = 4.607 - 0.110\ I$	$P = 5.992 - 0.448\ I$
	$I = 1.398 - 0.127\ P$	$I = 5.200 - 0.846\ P$
Line 98:	$P = 4.099 + 0.035\ I$	$P = 5.984 - 0.392\ I$
	$I = 0.114 + 0.117\ P$	$I = 6.559 - 1.084\ P$
Line 139:	$P = 4.005 + 0.034\ I$	$P = 5.958 - 0.558\ I$
	$I = -2.038 + 0.541\ P$	$I = 2.795 - 0.457\ P$
Line 143:	$P = 4.060 + 0.019\ I$	$P = 5.924 - 0.408\ I$
	$I = 0.047 + 0.059\ P$	$I = 1.732 - 0.249\ P$

The mean number of intercalary bundles associated with given numbers of primary double bundles and the theoretical means as given by the regression straight lines are shown on diagram 1.

For the normal plants of lines 75, 93, and 139 the agreement between the observed means and the regression line is very satisfactory. In line 98 a single seedling with 8 primary double bundles and 4 intercalary bundles gives a positive sign to the correlation and makes the agreement of theoretical and empirical means very poor indeed. In lines 139 and 143 the correlation is also positive. It must be noted that we are dealing here with a very narrow range of both primary double bundles and intercalary bundles, and with very small frequencies in some of the classes.

For the abnormal plants the agreement of empirical means and theoretical lines is apparently very poor indeed. This is perhaps largely attributable to two facts:

(*a*) The frequencies of primary double bundles are, practically speaking, concentrated in two classes, 5 and 6 bundles. From 93 to 99 percent of the seedlings fall in these two classes. As a result of this condition, the obtaining of trustworthy averages for the extreme classes of primary double bundles is, practically speaking, impossible.

(*b*) The influence of the two principal classes (5 and 6) of primary double bundles is such as to throw the theoretical mean number of intercalary bundles for higher classes of primary double bundles on the negative side of 0 in four of the five cases.

As a consequence, the actual mean number of intercalary bundles must lie above the line in all cases in which more than 6 primary double bundles are formed.

Whether these irregularities represent a significant deviation from linearity can be determined only when far larger series of data are available.

While the primary double bundles must probably be regarded as more fundamental structures than the intercalary bundles, it seems of interest to determine the mean number of primary double bundles associated with each number of intercalary bundles.

The lines for the regression of number of primary double bundles on number of intercalary bundles are represented with the empirical means on diagram 2. These figures show that, with the exception of the normal plants of lines 98, 139, and 143, the number of primary double bundles decreases slightly as the number of intercalary bundles increases. The rate of decrease is somewhat greater in the abnormal than in the normal plants.

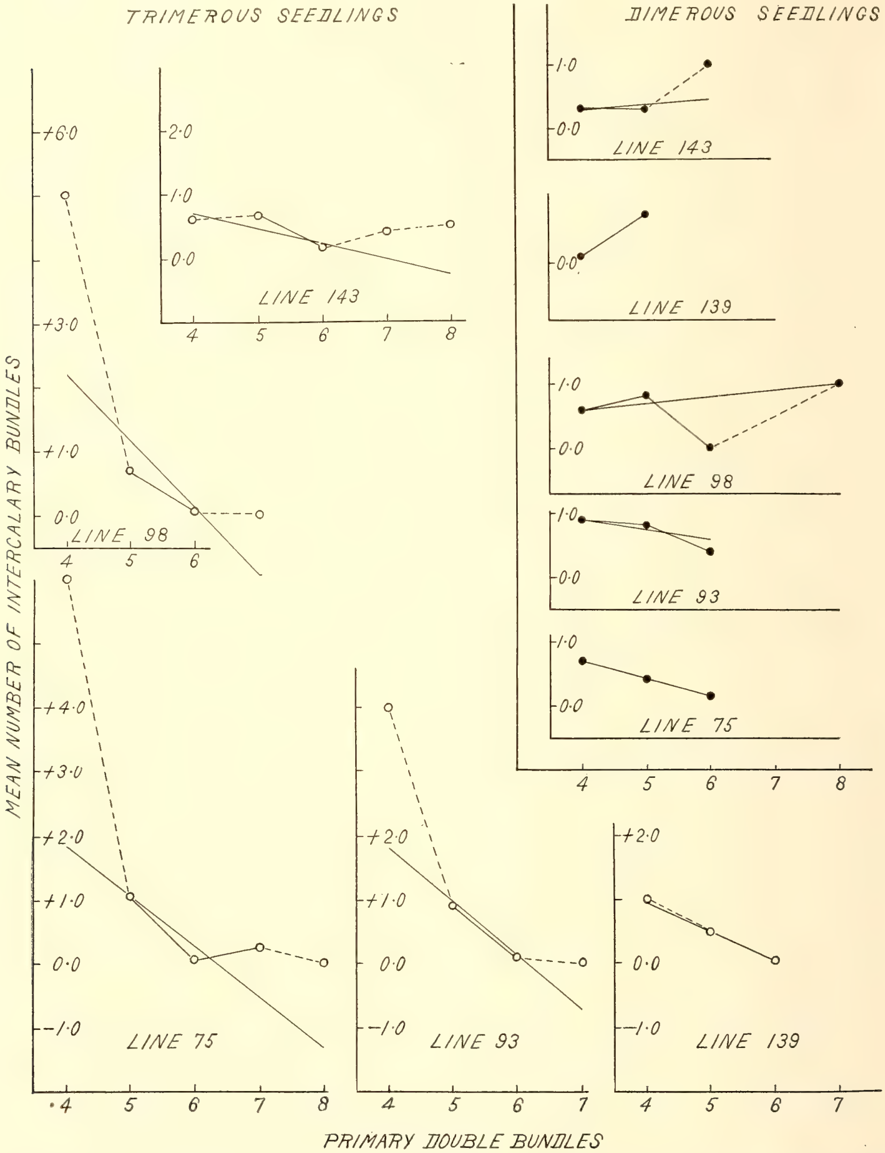


DIAGRAM 1. Regression of number of intercalary bundles on number of primary double bundles, at base of hypocotyl.

It is suggestive to note that the negative correlation between number of primary double bundles and number of intercalary bundles demonstrated here within seedlings of one class with regard to external structure is also evident when we pass from a type of seedling with a smaller to one with a higher number of primary double bundles. It has been shown in an earlier paper that (*a*) the number of trimerous seedlings having intercalary bundles is generally smaller than the number of dimerous seedlings with these accessory structures, and that (*b*) the average number of intercalary bundles is generally smaller in trimerous than in dimerous seedlings.

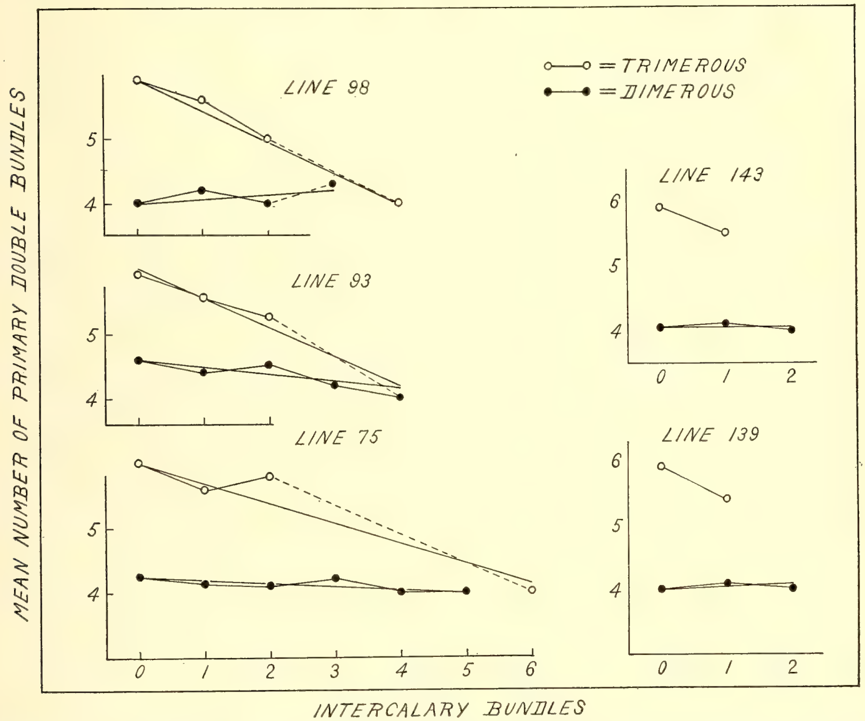


Diagram 2. Regression of number of primary double bundles on number of intercalary bundles, at base of hypocotyl.

2. *Relationship between the Total Number of Bundles and the Number of Bundles of the Two Types.* We may now inquire to what extent the variation in the total number of bundles depends upon the primary double bundles and to what extent upon the number of intercalary bundles. As a first step we determine the correlation between the total number of bundles and the number of primary double bundles, and between the total number of bundles and the number of intercalary bundles. These results are set forth in table 2.

We note that the correlations between the total number of bundles and the number of intercalary bundles are in all cases high, ranging from +.42

to +.80 in the trimerous and from +.76 to +.97 in the dimerous plants. The correlations for the dimerous plants are in all five cases slightly higher than those for the trimerous plants.

Table 2. *Comparison of Correlation between Total Bundles and Primary Double Bundles, r_{bp}, and between Total Bundles and Intercalary Bundles, r_{bi}, at Base of Hypocotyl*

	Trimerous			Dimerous			Difference	$\frac{\text{Diff.}}{E_{\text{diff.}}}$
	N	r	$\frac{r}{E_r}$	N	r	$\frac{r}{E_r}$		
Line 75								
r_{bi}	142	+.7788±.0222	35.1	142	+.8872±.0120	73.9	−.1084±.0245	4.42
r_{bp}	142	+.1532±.0552	2.77	142	+.3536±.0495	7.14	−.2004±.0741	2.70
$r_{bi}-r_{bp}$.		+.6256±.0591	10.6		+.5336±.0510	10.5		
Line 93								
r_{bi}	155	+.6934±.0281	24.7	155	+.7628±.0226	33.8	−.0694±.0361	1.92
r_{bp}	155	+.1409±.0531	2.65	155	+.5292±.0390	13.6	−.3883±.0655	5.92
$r_{bi}-r_{bp}$.		+.5525±.0600	9.21		+.2336±.0447	5.23		
Line 98								
r_{bi}	183	+.8001±.0179	44.6	183	+.8833±.0109	81.0	−.0832±.0200	4.16
r_{bp}	183	−.0664±.0496	1.34	183	+.5245±.0361	14.5	−.5909±.0616	9.59
$r_{bi}-r_{tp}$.		+.8665±.0529	16.4		+.3588±.0374	9.59		
Line 139								
r_{bi}	106	+.4203±.0539	7.79	305	+.9721±.0021	457.4	−.5518±.0539	10.2
r_{bp}	106	+.5707±.0422	12.9	305	+.3649±.0335	10.9	+.2058±.0555	3.71
$r_{bi}-r_{bp}$.		−.1504±.0697	2.16		+.6072±.0336	18.1		
Line 143								
r_{bi}	221	+.4382±.0367	11.9	420	+.8715±.0079	110.2	−.4333±.0375	11.55
r_{bp}	221	+.7126±.0223	31.9	420	+.5196±.0240	21.6	+.1930±.0328	5.88
$r_{bi}-r_{bp}$.		−.2744±.0429	6.40		+.3519±.0253	13.9		

The correlation between the total number of bundles and the number of primary double bundles is in general much lower. In line 98 the coefficient actually has the negative sign in the trimerous series. The differences between the correlation coefficients for total bundles and intercalary bundles, and for total bundles and primary double bundles, range from −.27 to +.87 in the trimerous plants and from +.23 to +.61 in the dimerous plants.

It is clear that the two types of plants differ rather fundamentally in this correlation. The correlation between the total bundles and the primary double bundles is very low in the trimerous plants. It is a much more substantial value in the dimerous plants.

Pursuing this point one step farther, we may determine by a special formula the relationship between the total number of bundles and the deviation of the number of intercalary bundles from the number which would be expected if the number of primary double bundles and intercalary bundles were in proportion to the total number of bundles formed.

Determining the correlation between the total number of bundles, b, and the deviation of the number of intercalary bundles, i, from their probable value by the formula[5]

$$r_{bz} = \frac{r_{bi} - r_b/r_i}{\sqrt{1 - r_{bi}^2 + (r_{bi} - r_b/r_i)^2}}$$

$$\text{where } z = i - \frac{\bar{i}}{\bar{b}} b,$$

we have the values given in table 3.

TABLE 3. *Correlation between Total Bundles at Base of Hypocotyl and Deviation of Number of Intercalary Bundles from Their Probable Number*

Line	Trimerous			Dimerous			Difference	$\frac{\text{Diff.}}{E_{\text{diff.}}}$
	N	r	$\frac{r}{E_r}$	N	r	$\frac{r}{E_r}$		
75	142	.7643±.0235	32.5	142	.8513±.0156	54.6	−.0870±.0283	3.07
93	155	.6787±.0292	23.2	155	.6693±.0299	22.4	+.0094±.0412	0.22
98	183	.7944±.0184	43.2	183	.8433±.0144	58.6	−.0489±.0224	2.18
139	106	.4066±.0546	7.45	305	.9701±.0023	421.8	−.5636±0.546	10.3
143	221	.3841±.0386	9.95	420	.8510±.0090	94.6	−.4669±03.96	11.8

The coefficients are positive and high, and very consistent for the two types of seedlings. They show that within one morphological type of seedling[6] an increase in the total number of bundles is primarily due to the formation of intercalary bundles, rather than to variation in the number of primary double bundles, although both types of bundles contribute to the end result.

SUMMARY

An investigation of the interrelationship of the numbers of primary double bundles, intercalary bundles, and total bundles (primary double bundles plus intercalary bundles) at the base of the hypocotyl in dimerous and trimerous seedlings of *Phaseolus vulgaris* leads to the following results:

1. In the trimerous seedlings there is a negative correlation of about medium value ($r = -.5 \pm$) between the number of primary double bundles and the number of intercalary bundles. Thus the number of intercalary bundles is smaller in seedlings with larger numbers of primary double bundles and *vice versa*. In dimerous seedlings the correlation is perhaps also negative in sign, but practically zero numerically.

---

[5] Harris, J. Arthur. The correlation between a variable and the deviation of a dependent variable from its probable value. Biometrika 6: 438–443. 1909; also, Further illustrations of the applicability of a coefficient measuring the correlation between a variable and the deviation of a dependent variable from its probable value. Genetics 3: 328–352. 1918.

[6] The differentiation of trimerous and dimerous seedlings has been shown to be due primarily to an increase in the number of primary double bundles.

This result for seedlings of the same morphological type is suggestive in its relation to the results of a comparison of seedlings which are externally dimerous and trimerous, since in general trimerous seedlings show an increase in number of primary double bundles but a decrease in number of intercalary bundles as compared with dimerous seedlings. As a result of this numerical compensation, most conspicuously evident in the trimerous seedlings, the total number of bundles shows a lower variability than it would if the numbers of the two types of bundles were quite independent.

2. The correlation between the total number of bundles (primary double bundles plus intercalary bundles) and the number of intercalary bundles is high. The coefficients for the dimerous seedlings are somewhat higher than those for the trimerous seedlings. The correlation between the total number of bundles and the number of primary double bundles is generally much lower. The correlation between the total number of bundles and the deviation of the number of intercalary bundles from that which would be expected if they occurred in the same proportionate frequency throughout the entire range of total bundle number is positive in sign and substantial in magnitude. In both types of seedlings variation in the number of intercalary bundles is therefore an important factor in determining variation in the total number of bundles at the base of the hypocotyl.

AREA OF VEIN-ISLETS IN LEAVES OF CERTAIN PLANTS AS AN AGE DETERMINANT

M. R. Ensign

(Received for publication February 26, 1921)

The available evidence in support of a theory of senescence in plants is very meager. The work of Minot (7), Child (2), Hertwig (5), Conklin (3) and others establishes quite definitely that complex animal forms are subject to a gradual retardation of physiological functions; also, that this retardation begins in the embryo and continues with more or less acceleration until death ensues.

Benedict (1) has attempted to show that plants are subject to similar changes of physiological functions. These, he claims, are initiated immediately after fertilization and are registered in the increasing complexity of the vascular ramifications in the leaves of certain dicotyledonous plants. In other words, the relative age of such perennial plants as vines, trees, and shrubs can be detected by determining the relative area of the "tissue islands" or vein-islets formed by the intersecting veins surrounding them. Old or senile plants, therefore, produce leaves whose vein-islets are smaller in area than those in leaves of young plants of the same species grown under similar environmental conditions.

Beginning with these premises, the writer (4) studied the venation of leaves produced by polyembryonic Citrus seedlings (*Citrus grandis*). During the progress of this work certain questions arose regarding the accuracy of the methods employed by Benedict in determining the area of vein-islets in the leaves which he used. In order to shed some light upon this point the work herein reported was undertaken. The data collected are not as extensive as might be desired, but inasmuch as further investigation had to be postponed indefinitely, they are presented for what they may be worth.

Methods and Materials

Leaves from the following plants were studied with reference to their venation: *Berberis vulgaris* L., *Berberis Thunbergii* DC., *Castanea dentata* Borkh., *Quercus alba* L., *Fagus caroliniana* Fernald and Rehder, *Vitis vulpina* L., and an undetermined species of Vitis growing in the physiological greenhouse at Cornell University, Ithaca, New York. The trees and vines grew in the immediate vicinity of Ithaca, and the barberries grew on the university campus.

In collecting the leaves from these plants the trunk diameters were taken as an index of relative age. For comparison two or more plants growing in the same habitat were selected whose trunk diameters were indicative of youth and old age respectively. Leaves which had approximately equal light exposure were taken from these plants for study.

Only mature leaves were used, inasmuch as it has been shown by Benedict (1) and by the writer that the area of vein-islets in immature leaves is less than that of vein-islets in mature leaves of the same species.

From five to fifteen leaves from each plant were selected and taken to the laboratory. Portions of each leaf were cleared and stained, and determinations of the size of vein-islets were made according to a previously described method (4). At least four determinations were made from different places[1] on the same leaf. Thus, from each plant from twenty to seventy-five determinations of vein-islet areas were made in order to reduce to a minimum the probable error due to variation.

The method used by Benedict in determining the area of vein-islets is as follows:

> The collected leaves were taken immediately to the laboratory, measured as to length, breadth, and area, and weighed. The venation was then photographed in the following way: a heavy black paper was pasted to a clean glass plate, four by five inches in size. Ten openings, approximately four by ten millimeters in size, were then cut in the black paper. From the same part of each leaf pieces a little larger than the openings were cut, and these were laid over the openings, so that each of the ten leaves was represented. A clear glass plate was then laid over all, and the whole was bound together by elastic bands, placed in the negative holder of an enlarging camera, and photographed at an enlargement of three diameters. Negatives showing the veinlets clearly were obtained after some practice, and from these negatives velox prints were made. . . . The counting (of the number of vein-islets in the opening) was done under a lens, and a sharp needle was used to prick each vein-islet as it was counted on the photograph.

It occurred to the writer that such a method might be conducive to inaccuracy for the following reasons: Leaves growing on the same plant and even on the same twig vary greatly in shape and size. There is no good reason, therefore, for expecting to find such characters as leaf thickness and chlorophyll content constant. In fact, the most casual observation soon discloses the fallacy of such a premise. This being the case, the proportion of vascular bundles visible in the uncleared leaves would vary directly with the leaf thickness and the chlorophyll content.

Only one determination from each leaf appears to be entirely inadequate to overcome the probable error. It would also appear that the inaccuracy would be exaggerated by each step in the photographing, developing, and printing processes, especially when the magnification used was only three diameters.

Experimental Data

In order to find what percentage of the vascular tissue is hidden by chlorophyll, leaves growing under as nearly identical environmental conditions as possible were selected. Mature leaves from an unidentified species of grape (*Vitis* sp.) growing in the plant physiological greenhouse at Cornell furnished the necessary material. Only mature leaves were used, since it has been shown that the area of the vein-islets in immature leaves is much less than that in mature leaves.

---

[1] Benedict (1) and the writer (4) have shown that the sizes of vein-islets are quite constant in various places in a single leaf.

Portions of each of thirty-eight leaves from a single plant, having the same light exposure, were examined under the projection apparatus, as described in a previous article (4). The magnification used was thirty-eight diameters. A similar portion of each leaf was cleared and stained and determinations of the vein-islet area were made with the same apparatus and at the same magnification. Figure 3, Plate XXIII, shows the vascular tissue of the cleared and stained portion of this grape leaf. Table 1 shows a summary of the data obtained from this study. It is evident from this study that many vein-islets are invisible in the uncleared leaves even with a magnification more than twelve times as great as that used by Benedict. Indeed, the average shows that nearly half the vascular tissue is hidden by the chlorophyll, and in some leaves as much as sixty-two percent is invisible. On the other hand, some leaves show the major portion of their vascular tissue in the uncleared, unstained condition.

TABLE 1. *Relative Size of Vein-islets of Uncleared and Cleared Portions of the Same Leaf (Vitis sp.)*

Number of Leaf	Uncleared Portion		Cleared Portion		Vein-islets Hidden in Uncleared Leaves (percent)
	No. Vein-islets in Unit Area (4 sq. mm.)	Area Vein-islets (sq. mm.)	No. Vein-islets in Unit Area (4 sq. mm.)	Area Vein-islets (sq. mm.)	
1	14	.2860	30	.1333	54
2	14	.2860	34	.1176	59
3	21	.1904	33	.1212	37
4	13	.3200	34	.1176	62
5	18	.2222	35	.1143	49
6	25	.1600	30	.1333	17
7	17	.2353	33	.1212	48
8	21	.1904	35	.1143	40
9	15	.2666	30	.1333	50
10	12	.3200	29	.1379	59
11	16	.2500	29	.1379	39
12	17	.2353	31	.1290	46
Average	16.9	.2468	32	.1259	47.5
Mean Average 38 Leaves	16.9±.135	.2485	32±.263	.1240	47.5%

Benedict (1) presents data which show that the vein-islets in uncleared leaves grown in the shade are smaller than those in leaves exposed to direct sunlight. These data interpreted in the light of the experimental results shown in table 1 mean, no doubt, that the leaves grown in the direct light were thicker and contained a larger amount of chlorophyll, so that fewer veins were visible in a unit area. Schuster (8) records a similar condition in the leaves of *Ampelopsis Veitchii.*

Inasmuch as Benedict found a direct correlation between age differences and vein-islet areas in leaves from various perennial plants, some of these plants were studied by clearing and staining. The leaves of *Fagus caroliniana* were taken from plants growing in close proximity to each other yet having large differences in trunk diameters. A summary of the data from this study is presented in tables 2 and 3. Although Benedict did not study the leaves of the beech, he intimates that the leaves of all the woody perennial plants show senescence by the constantly increasing amount of vascular tissue in their leaves as they increase in age. The data in tables 2 and 3 do not show distinctive differences.

TABLE 2. *Relative Size of Vein-islets of Leaves of Fagus caroliniana Fernald and Rehder, from Trees of Different Ages*

Number of Leaf	Trunk Diameter 5.4 cm.		Trunk Diameter 22 cm.	
	No. Vein-islets in Unit Area (4 sq. mm.)	Area Vein-islets (sq. mm.)	No. Vein-islets in Unit Area (4 sq. mm.)	Area Vein-islets (sq. mm.)
1	63	.0635	69	.0580
2	62	.0645	64	.0625
3	66	.0606	60	.0666
4	63	.0635	61	.0655
5	64	.0625	65	.0615
6	63	.0635	62	.0645
7	61	.0655	62	.0655
8	63	.0635	63	.0635
9	60	.0666	—	——
10	64	.0625	—	——
Average	62.9	.0637	63.2	.0635

TABLE 3. *Comparison of Size of Vein-islets in Relation to Age of Tree in Fagus caroliniana*

Diameter of Trunk (cm.)	6.2	7.5	18	20
Area of Vein-islets (10 Leaves) (sq. mm.)	.0678	.0640	.0641	.0625

In leaves from specimens of *Castanea dentata* having a trunk-diameter difference of 49.5 cm., Benedict records a difference in vein-islet area of 0.3 square millimeter. In table 4 a summary of the findings in cleared and stained leaves is shown. Here there is but the very slightest difference shown in the area of the vein-islets. The variation found in individual leaves from the same tree shows as great differences.

TABLE 4. *Relation of Size of Vein-islets to Age in Castanea dentata Borkh.*

Diameter of Trunk (cm.)	5.2	3.7	50.4	38
Average Area of Vein-islets of 15 Leaves (sq. mm.)	.0897	.0697	.0876	.0677

A number of determinations made from the cleared leaves of white oak (*Quercus alba*) and of *Platanus occidentalis* revealed no correlation between the size of vein-islets and their relative ages.

The barberry leaves studied were from plants of known age. The department of landscape gardening[2] at Cornell University had several hundred one-year-old seedlings in cultivation. In several places on the campus, barberry plants were known to have been growing from six to twelve years, and were probably several years old when first planted. Leaves from these plants of different ages were cleared, stained, and studied as to venation. A summary of these determinations is presented in tables 5 and 6.

TABLE 5. *Relation of Size of Vein-islets to Age in Berberis vulgaris L.*

Known Age of Plants	1 year	6 years +
Average Area of Vein-islets of 35 Leaves (sq. mm.)...........	.2405	.2378

TABLE 6. *Relation of Size of Vein-islets to Age in Berberis Thunbergii DC.*

Known Age of Plants	2 years	12 years +
Average Area of Vein-islets of 18 Leaves (sq. mm.)...........	.2163	.2196

The conclusions that may be drawn from these results are subject to two interpretations. (1) The age differences may not be sufficiently great to influence very materially the size of the vein-islets. Yet Benedict records instances in which individuals of *Vitis vulpina* with an age difference of not more than three to five years show a positive correlation. (2) It may be that this particular perennial does not register its relative age in its more or less complex nervature. Figures 1 and 2, Plate XXIII, show the nature of venation of *Berberis vulgaris.*

The work up to this point was done while at Cornell in 1916–17. The data presented below were obtained from leaves of *Vitis vulpina* which were gathered from various places near Ithaca, New York. They were preserved in 85 percent alcohol in test tubes until December, 1918, and were in very good condition. Because of the fact, however, that most of the chlorophyll had been extracted, a comparison between the sizes of vein-islets in cleared and uncleared material was not possible.

The plants from which these leaves were taken were selected and marked in the same manner as that described by Benedict (1). The greatest care was taken to secure leaves for comparison that were growing under as nearly similar environmental conditions as possible. Data from cleared and stained leaves only are given in tables 7–12. The results show quite wide variations as to size of vein-islets in the leaves from different vines. The significant fact to be noted, however, is that there is no definite correlation

---

[2] Courtesy of Professor Hunn.

Table 7. *Relation of Size of Vein-islets to Age in Leaves of Vitis vulpina L., Vines 1 and 2*

Number of Leaves	Vine 0.8 cm. Diameter (5 Annual Rings)		Vine 5.2 cm. Diameter (17 Annual Rings)	
	No. Vein-islets in Unit Area (2.25 sq. mm.)	Area Vein-islets (sq. mm.)	No. Vein-islets in Unit Area (2.25 sq. mm.)	Area Vein-islets (sq. mm.)
12	17.4	.1421	16.6	.1389

Table 8. *Relation of Size of Vein-islets to Age in Leaves of Vitis vulpina L., Vines 3 and 4*

Number of Leaf	Vine 1.3 cm. Diameter (6 Annual Rings)		Vine 10 cm. Diameter (25 Annual Rings)	
	No. Vein-islets in Unit Area (2.25 sq. cm.)	Area Vein-islets (sq. cm.)	No. Vein-islets in Unit Area (2.25 sq. cm.)	Area Vein-islets (sq. cm.)
1	19	.1184	20	.1125
2	17	.1323	21	.1071
3	18	.1250	20	.1125
4	19	.1184	18	.1250
5	18	.1250	17	.1323
6	17	.1323	19	.1184
7	20	.1125	16	.1531
8	19	.1184	17	.1323
9	20	.1125	16	.1531
10	21	.1071	20	.1125
11	20	.1125	17	.1323
12	17	.1323	18	.1250
Average	18.7	.1205	18.2	.1270

Table 9. *Relation of Size of Vein-islets to Age in Leaves of Vitis vulpina L., Vines 5 and 6*

Number of Leaves	Vine 1.3 cm. Diameter (6 Annual Rings)		Vine 5.2 cm. Diameter (17 Annual Rings)	
	No. Vein-islets in Unit Area (2.25 sq. mm.)	Area Vein-islets (sq. mm.)	No. Vein-islets in Unit Area (2.25 sq. mm.)	Area Vein-islets (sq. mm.)
10	13	.1740	16.6	.1389

Table 10. *Relation of Size of Vein-islets to Age in Leaves of Vitis vulpina L., Vines 7 and 12*

Number of Leaves	Vine 3 cm. Diameter (12 Annual Rings)		Vine 6.4 cm. Diameter (18 Annual Rings)	
	No. Vein-islets in Unit Area (2.25 sq. mm.)	Area Vein-islets (sq. mm.)	No. Vein-islets in Unit Area (2.25 sq. mm.)	Area Vein-islets (sq. mm.)
10	21.8	.1054	18.9	.1192

TABLE 11. *Relation of Size of Vein-islets to Age in Leaves of Vitis vulpina L., Vines 9 and 10*

Number of Leaves	Vine 1 cm. Diameter (5 Annual Rings)		Vine 4 cm. Diameter (15 Annual Rings)	
	No. Vein-islets in Unit Area (2.25 sq. mm.)	Area Vein-islets (sq. mm.)	No. Vein-islets in Unit Area (2.25 sq. mm.)	Area Vein-islets (sq. mm.)
12	21.8	.1042	25.2	.0898

TABLE 12. *Relation of Size of Vein-islets to Age in Leaves of Vitis vulpina L., Vines 11 and 12*

Number of Leaves	Vine 1 cm. Diameter (5 Annual Rings)		Vine 6.4 cm. Diameter (18 Annual Rings)	
	No. Vein-islets in Unit Area (2.25 sq. cm.)	Area Vein-islets (sq. mm.)	No. Vein-islets in Unit Area (2.25 sq. mm.)	Area Vein-islets (sq. mm.)
12	17	.1342	18.9	.1192

between vein-islet area and age differences. The greatest variation was found in the leaves from vines 9 and 10 (table 10), where the average area of vein-islets showed a difference of 0.0144 sq. mm. The age difference here was ten years according to the number of annual rings. In contrast to these figures, the data in table 8 are interesting. In this case there is an age difference of nineteen years but a difference of but 0.0065 sq. mm. in the average area of vein-islets, the smaller islets being found in the younger plant. For a similar age difference Benedict records a difference in vein-islet area of 0.2553 sq. mm.

It is interesting to make some other comparisons between the sizes of vein-islets as found by Benedict and those obtained by the writer from leaves of the same species, *Vitis vulpina* (table 13). Of course this com-

TABLE 13. *Comparison of Vein-islet Areas Obtained from Individuals of Varying Age but of the Same Species (Vitis vulpina L.)*

Benedict		The Writer	
Number Annual Rings	Vein-islet Area (sq. mm.)	Number Annual Rings	Vein-islet Area (sq. mm.)
5	0.4845	5	0.1421
6	0.3983	6	0.1740
6	0.3684	6	0.1205
6	0.3983	5	0.1042
11	0.3690	12	0.1054
16	0.2966	15	0.0898
17	0.3310	17	0.1389
16	0.2966	17	0.1389
17	0.3160	18	0.1192
25	0.2503	25	0.1270
Average	0.3509		0.1260

parison is not so instructive as one made from cleared and uncleared leaf portions taken from paired plants and studied by the same methods. However, these data compare favorably with the results obtained from the study of the cleared and uncleared leaves of the undetermined species of Vitis shown in table 1. It will be noted that in both cases the uncleared leaves show vein-islet areas from two to three times larger than those from cleared and stained leaf portions.

Discussion

The above data show that any study of leaf venation made from uncleared leaves is wholly unreliable. The varying thickness and chlorophyll content of leaves render many of the smaller veins entirely invisible. Furthermore, some unpublished results obtained by Heinicke[3] do not corroborate the preliminary venation studies of uncleared apple leaves made by Benedict. Heinicke finds no correlation between vein-islet area and the age of a large number of apple varieties. These are of known age, *i.e.*, it is known when they originated as seedlings.

The results herein presented do not show a single instance in which the leaf venation might be taken as an index of the relative ages of the plants in question. While working with yearling Citrus seedlings, a number of grape-fruit leaves were obtained from some of the oldest trees in the vicinity of Miami, Florida, and it was found that the venation of the leaves from the yearling plants was identical with that from the older trees. A similar condition was found in regard to the venation of some orange leaves taken from a plant growing in the Sage greenhouse at Cornell. This plant was probably ten or fifteen years of age. These similarities in venation seem to be indicative of something more than mere coincidences.

As intimated at the beginning, it is highly desirable that more data be secured bearing upon this problem. There are certain phases which require more elucidation before satisfactory conclusions can be derived. It may be that the venation of the uncleared leaves of *Vitis vulpina* and other plants with which Benedict worked shows some correlation with age which the cleared leaves fail to reveal. Such a possibility, however, does not seem tenable.

Just as this goes to press the following statement comes from August Henry, Royal Society of Dublin:

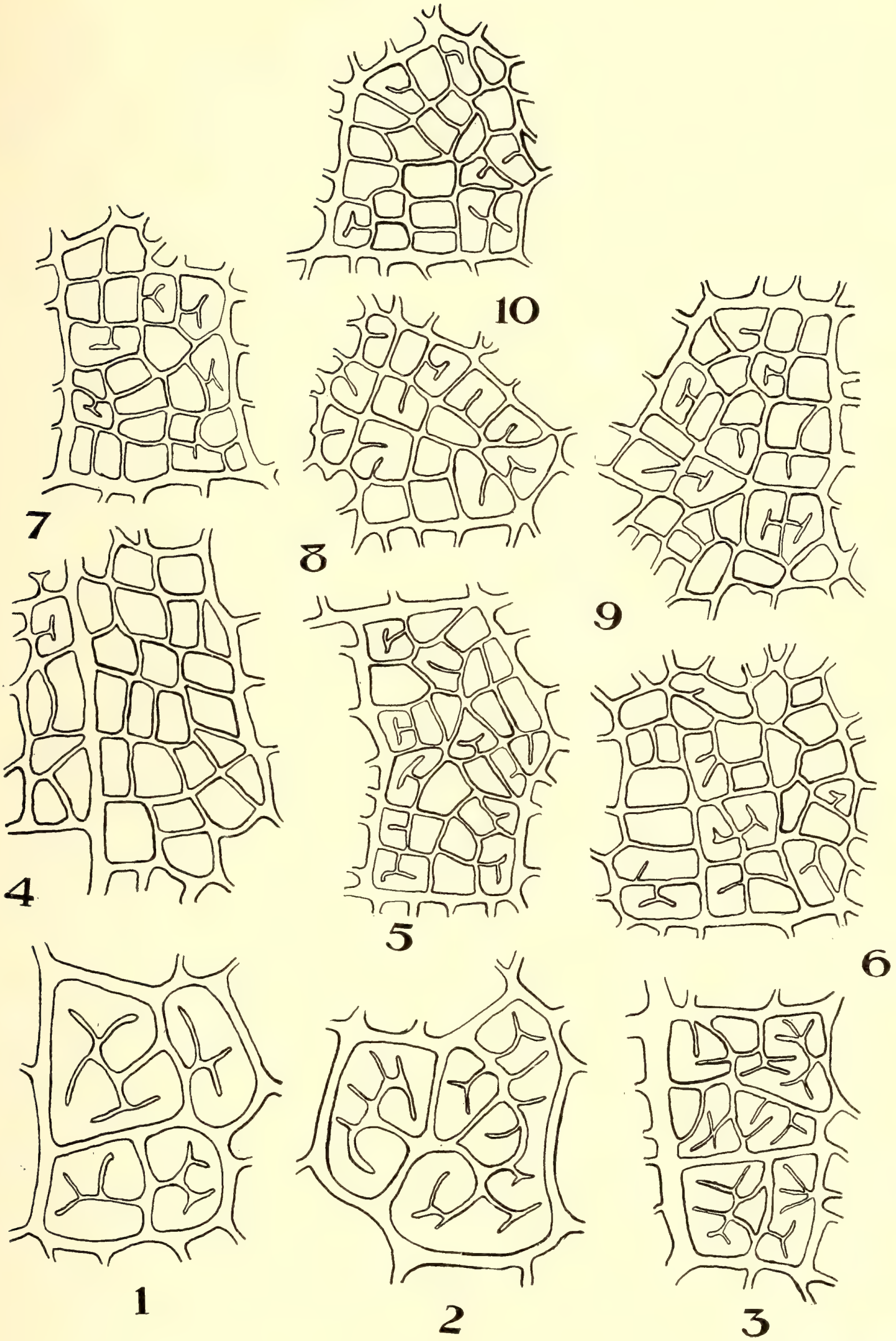
> I tried this [venation vs. age] in the various species and hybrids while working on my paper on "The Origin of the London Kane." In this I dealt with the genus Platanus (*Proc. Royal Irish Acad.*) without any very conclusive results. Here the question lay in regard to whether trees produced of cuttings were as old as the original, or only as young as the time the cuttings were started.

Summary and Conclusions

1. From seventeen to sixty-two percent of the vein-islets are invisible in uncleared leaves of *Vitis* sp.

---

[3] A. J. Heinicke, assistant professor of horticulture, Cornell University.


10
7
8
9
4
5
6
1
2
3

Ensign: Vein-islet Area in Leaves.

2. No correlation was evident between the age of the following plants as indicated by their trunk diameters and the vein-islet area of their leaves: *Fagus caroliniana* Fernald and Rehder; *Castanea dentata* Borkh.; *Berberis vulgaris* L.; *B. Thunbergii* DC., and *Vitis vulpina* L.

BIBLIOGRAPHY

1. **Benedict, H. M.** Senile changes in leaves of *Vitis vulpina* L. and certain other plants. New York Agr. Exp. Sta. (Cornell) Memoir **7**: 281–370. 1915.
2. **Child, C. M.** The process of reproduction in organisms. Biol. Bull. **23**: 1–39. 1912.
   Senescence and rejuvenescence. pp. 481. Chicago, 1915.
3. **Conklin, E. G.** Cell size and nuclear size. Jour. Exp. Zool. **12**: 1–98. 1912.
   The size of organisms and their constituent parts in relation to longevity, senescence and rejuvenescence. Pop. Sci. Mo. **83**: 178–198. 1913.
4. **Ensign, M. R.** Venation and senescence of polyembryonic Citrus plants. Amer. Jour. Bot. **6**: 311–329. 1919.
5. **Hertwig, R.** Ueber Korrelation von Zell- und Kerngrösse und ihre Bedeutung für die geschlechtliche Differenzirung und die Teilung der Zelle. Biol. Centralb. **23**: 194–223. 1903.
6. **Jennings, H. S.** Age, death and conjugation in the light of the work on lower organisms. Pop. Sci. Mo. **80**: 563–577. 1912.
   Effect of conjugation in Paramecium. Jour. Exp. Zool. **14**: 279–391. 1913.
7. **Minot, C. S.** The problem of age, growth and death. pp. 280. New York, 1908.
8. **Schuster, W.** Die Blattaderung des Dicotylenblattes und ihre Abhängigkeit von äusseren Einflussen. Ber. Deutsch. Bot. Ges. **26**: 194–237. 1908.

EXPLANATION OF PLATE XXIII

All figures are drawings made from the projection of the cleared and stained leaves. × 38.

FIG. 1. Venation of a leaf from 6-year-old barberry (*Berberis vulgaris*).

FIG. 2. Venation of a leaf from 1-year-old barberry (*Berberis vulgaris*).

FIG. 3. Venation of a mature leaf from undetermined species of Vitis.

FIG. 4. Venation of grape leaf (*Vitis vulpina*) having a trunk diameter of 1.3 cm. and showing 6 annual rings.

FIG. 5. Venation of a leaf from chestnut (*Castanea dentata*) having a trunk diameter of 38 cm.

FIG. 6. Venation of a leaf of grape (*Vitis vulpina*) having a trunk diameter of 10 cm. and showing 25 annual rings.

FIG. 7. Venation of a leaf from grape (*Vitis vulpina*) having a trunk diameter of 0.8 cm. and showing 5 annual rings.

FIG. 8. Venation of a leaf from grape (*Vitis vulpina*) having a trunk diameter of 5.2 cm. and showing 17 annual rings.

FIG. 9. Venation of a leaf from grape (*Vitis vulpina*) having a trunk diameter of 6.4 cm. and showing 18 annual rings.

FIG. 10. Venation of a leaf from chestnut (*Castanea dentata*) having a trunk diameter of 3.7 cm.

UNUSUAL RUSTS ON NYSSA AND URTICASTRUM[1]

E. B. Mains

(Received for publication February 25, 1921[2])

During the past year, two very interesting rusts of the family Melampsoraceae have come to the writer's attention. The first of these, upon *Nyssa aquatica*, has remained in the form genus Uredo since its description in 1890 by Ellis and Tracy under the name of *Uredo Nyssae*. While preparing the manuscript of this species for the North American Flora, the writer was fortunate in discovering the telia. A study of this stage shows that the species can not be placed in any established genus and in consequence the following genus is proposed.

Aplopsora[3] gen. nov.

Cycle of development imperfectly known, only uredinia and telia recognized, both subepidermal.

Uredinia pulverulent; urediniospores produced singly, echinulate, the pores obscure.

Telia lenticular, at first covered by the epidermis, soon becoming naked and cinereous from germination; teliospores one-celled, cylindric, in one layer, the wall thin, colorless, smooth, germinating shortly after reaching full size.

Aplopsora Nyssae (Ellis & Tracy) comb. nov.

Uredo Nyssae Ellis & Tracy. Jour. Myc. **6**: 77. 1890

O + I. Pycnia and aecia unknown.

II. Uredinia hypophyllous, scattered, round, minute, 0.1–0.3 mm. across, early naked, pulverulent, cinnamon-brown, ruptured epidermis inconspicuous; paraphyses peripheral, united below into a short, inconspicuous pseudoperidium, clavate, incurved, 16–26 μ long, the wall 1 μ thick, on convex side above up to 3–4 μ, brownish-yellow; urediniospores obovoid or oblong, 13–17 by 16–26 μ; wall yellow or pale cinnamon-brown, 1 μ, rather closely and finely echinulate, the pores obscure.

III. Telia hypophyllous, gregarious in small groups, round, small, 0.2–0.5 mm. in diameter, at first covered by the epidermis, soon becoming naked, very pale translucent yellow, becoming cinereous from germination; teliospores cylindric, 7–15 by 29–40 μ, rounded above and below, in one layer; wall colorless, very thin, 0.5 μ or less, uniform in thickness, smooth, soon germinating with typical, external basidia.

---

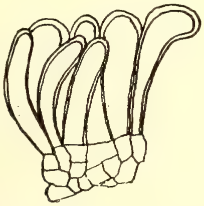
[1] Contribution from the Botanical Department of the Purdue University Agricultural Experiment Station. Read in part before the Mycological Section of the Botanical Society of America at Chicago, December 29, 1920.

[2] Culture results revised to July 1, 1921.

[3] From ἀπλόος, *simple*, and Ψώρα, *scab*, referring to the telium of one spore layer.

Nyssa aquatica L., Jackson, Miss., Oct. or Nov. 12, 1888, II & III, *S. M. Tracy 1200* (type)[4]; Ocean Springs, Miss., Nov. 4, 1891, II, *F. S. Earle;* Ocean Springs, Miss., Nov. 8, 1891, II, *F. S. Earle;* Great Cypress Swamp, Calvert City, Kentucky, II, *W. W. Eggleston;* obtained from a phanerogamic specimen, no. *5374*, in the herbarium of the New York Botanical Garden by H. S. Jackson.

The uredinia of this rust do not differ markedly from those of a number of genera belonging to the Melampsoraceae. The incurved paraphyses bordering the uredinium and united below into a short pseudoperidium are characteristics which are found in some species of Phakopsora (figs. 1, 2). The urediniospores are borne in a very similar manner to that described by Butler[5] for *Cerotelium Fici* (Cast.) Arth. (*Kuehneola Fici* Butler), the hymenium consisting of a mass of cubical cells upon the uppermost of which the spores are borne (fig. 2). The cells bearing the spores have little to distinguish them from the other cells of the hymenium except that they are separated somewhat from each other. Whether these cells are to be considered as pedicels or whether, as Butler suggests for *C. Fici*, they in turn may develop into spores, cannot be determined from the material at hand, but there is no evidence that the spores form chains.



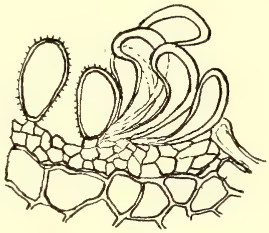


FIG. 1 (Left). Paraphyses of *Aplopsora Nyssae* showing short pseudoperidium at their bases.
FIG. 2 (Right). Portion of uredinium of *Aplopsora Nyssae* showing peripheral position of paraphyses and manner in which urediniospores are borne.

The telia on the other hand characterize this rust as generically distinct. The teliospores arranged in a one-layered crust of cylindric, one-celled teliospores would place this species in a group with Melampsora, Melampsoridium, and Chnoopsora (fig. 3). From the first two it is distinct tno only in uredinial characters but in that the telium soon ruptures the epidermis and the teliospores germinate at once. In these characters it is much like species of Chnoopsora but differs in the method of teliospore formation. In species of Chnoopsora the teliospores are produced over a period of time due to young sporogenous hyphae developing between the older ones and forming teliospores to replace those which have germinated; while in *Aplopsora Nyssae* the teliospores are all formed and matured at practically the same time without subsequent spore production.

---

[4] The description of Ellis gives the host as *N. capitata* and the date as Nov. 1888. Small, in his Flora of the Southeastern United States, gives the range of *N. capitata* as South Carolina to Georgia and Florida. Since other collections of this rust are on *N. aquatica*, it is likely that this collection is also on that host. There is also some confusion as to the date of the collection, some packets being labeled 10/12/1888 and some 11/12/1888.

[5] **Butler, E. J.** Notes on some rusts in India. Ann. Mycol. 12: 76–82. 1914.

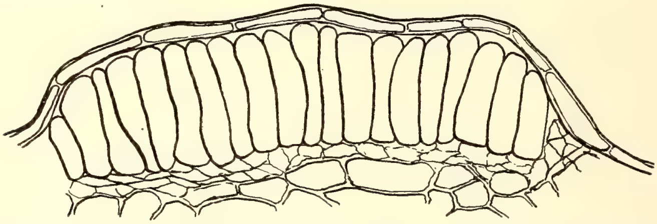


FIG. 3. Section through mature telium of *Aplopsora Nyssae* showing arrangement of spores in one layer.

Just what the complete life cycle of *Aplopsora Nyssae* may be is difficult to say. The early germination of the teliospores without a resting period, if this rust is autoecious, would apparently necessitate the production of pycnia and aecia or pycnia and uredinia immediately following infection or else the development of a systemic mycelium from which such stages would be produced the following spring. The herbarium material available does not show either condition and it is probable that this rust is heteroecious. The aecial stage and probable aecial hosts cannot be foretold definitely. The related genus, Melampsora, has for its aecial stage a Caeoma with either subcuticular or subepidermal pycnia. The alternate hosts belong to a number of genera, mostly, however, conifers. Chnoopsora has for its aecial stage a Caeoma with subcuticular pycnia, the species with known life cycle being autoecious. *Melampsoridium Betulae* (Schum.) Arth. has a peridermium with subcuticular pycnia on Larix. In consequence it would be expected that *Aplopsora Nyssae* would have a Caeoma or a Peridermium for its aecial stage, probably upon some conifer. Specimens of *Caeoma strobilina* Arth. on pine are in the Arthur herbarium from Gulfport and Agricultural College, Mississippi, which may possibly be the alternate stage of the Nyssa rust. Hedgcock and Hunt[6] have reported connecting this rust with a Uredo on Quercus from material collected in Florida. They, however, mention that some of the collections of *Caeoma strobilina* have pseudoperidia and that another rust is represented here, which is unconnected. There is also the possibility that this rust may produce a Peridermium on pine which is at present confused with one of the many species of Peridermium found in the south.

The second rust occurs on *Urticastrum divaricatum* and was received by Dr. J. C. Arthur from Prof. H. W. Anderson who wrote that he had collected it for a Synchytrium but upon examination found what he thought were uredinia. An examination of the material showed that Professor Anderson was correct in considering the fungus a rust and also disclosed that in addition to the uredinia abundant telia were present, most of which were white from the germination of the teliospores. A study of this rust showed that the telium consisted of a crust of one-celled, colorless teliospores, borne in chains of two or three. In these and other characters the rust seemed to belong in the genus Cerotelium. The characteristics and relationships of the rust all pointed to a heteroecious life cycle. Since infection would have to occur in the fall, it appeared quite likely that the aecial stage either developed upon biennial parts of the host such as the needles of some conifer or was systemic. Since conifers were not to be found in the vicinity of the Urticastrum rust at Urbana, it appeared more likely that the aecial stage was systemic. Dr. Arthur immediately suggested *Aecidium Dicentrae* Trel. as the likely aecial stage, since not only is this rust systemic but unlike the usual Aecidium of the region it possesses large, subcuticular pycnia, a characteristic of many of the Melampsoraceous rusts. This conclusion was greatly strengthened by discovering in the herbarium a specimen of the Aecidium collected by Professor Anderson in the same woods earlier in the season. The only apparently serious objection to this connection was the manner of growth of *Bicuculla Cucullaria* (L.) Millsp. (*Dicentra Cucullaria* Torr.). This plant develops and flowers early in the spring and then soon dies down, so that by the time teliospores of the Urticastrum rust are germinating, there is nothing of the Bicuculla plant above ground except an occasional corm. This connection would therefore necessitate an unusual type of infection. *Aecidium Dicentrae*, however, resembles so closely what it was felt the aecial stage should be that sowings were made by placing leaves bearing germinating teliospores of the Urticastrum rust on soil containing corms of *Bicuculla Cucullaria*. The pots of corms were placed out of doors during the winter and then brought into the greenhouse early the next spring. No infection appeared. In spite of this, it was still felt that the rusts of Urticastrum and of Bicuculla were connected. Another attempt was made this spring (1921) by sowing aeciospores of the Bicuculla rust sent from Urbana, Illinois, by Professor Anderson. This sowing produced typical uredinia upon *Urticastrum divaricatum*. The lack of results from the sowings of basidiospores upon the Bicuculla corms may have been due to the effect that the high temperature of the greenhouse had upon the development of the plants, since infected corms sent by Professor Anderson in the summer of 1920 when brought into the greenhouse this spring showed the rust in only a few cases and then only pycnia were produced.

---

[6] **Rhoads, A. S., Hedgcock, G. G., Bethel, E., and Hartley, C.** Host relationships of the North American rusts, other than Gymnosporangiums, which attack conifers. Phytopath. 8: 309–352. 1918.

Cerotelium Dicentrae (Trel.) Mains and Anderson comb. nov.

Aecidium Dicentrae Trel. Trans. Wis. Acad. Sci. **6**: 136. 1884.

O. Pycnia amphigenous, somewhat scattered, usually near the margin of the leaf, conspicuous, subcuticular, violet becoming dark chestnut or chocolate-brown, applanate or discoidal, 160–200 μ in diameter by 40–60 μ high; ostiolar filaments wanting.

I. Aecia hypophyllous, subepidermal, scattered over the entire leaf, cupulate, 0.1–0.5 mm. in diameter; peridium white, the margin remaining somewhat incurved, erose; peridial cells rhomboidal in side view, 15–20 by 24–35 μ, overlapping considerably, the outer wall 7–9 μ thick, faintly transversely striate, the inner wall 3–5 μ thick, closely and finely verrucose; aeciospores somewhat angularly globoid or ellipsoid, 12–17 by 13–21 μ; wall colorless, thin, 1 μ or less, closely and very finely verrucose.

Bicuculla Cucullaria (L.) Millsp. (*Dicentra cucullaria* Torr.), Pine Hills, Union Co., Ill., April 24, 1882, *A. B. Seymour 4252;* Madison, Wis., June, 1884, *L. H. Pammel;* Decorah, Ia., May, 1886, *E. W. D. Holway* (Barth. N. Am. Ured. *203*); May 18, 1887 (Sydow, Ured. *497*); Iowa City, Ia., May 7, 1887, *Thos. H. Macbride;* Morning Sun, Iowa, April 16, 1895, *Geo. W. Carver;* Manhattan, Kan., May, 1888 (Kellerm. & Swingle, Kan. Fungi *2*); Topeka, Kan., May 9, 1904, *H. W. Baker* (Ellis & Ev. Fungi Columb. *1903*); Oakwood, S. Dakota, May 9, 1891, *E. N. Wilcox;* Crawfordsville, Ind., June, 1893, *E. W. Olive;* Concordia, Mo., June 20, 1888, May, *C. H. Demetrio* (Rab.-Paz. Fungi Eur. *4335;* Nebraska, 1899, *A. A. Hunter;* Nebraska City, Nebr., April, 1899, *Thornber;* Lancaster, Pa., May 5, 1900, *A. A. Heller 4972;* New York City, Apr. 21, 1913, May 4, 1914, *F. D. Fromme;* Van Cortlandt Park, New York City, April 25, 1912, *F. D. Fromme;* April 20, 1915, *P. Wilson 52;* Williamsbridge, New York City, April 28, 1916, *P. Wilson 230;* vicinity of Grassy Sprain Reservoir, Westchester Co., N. Y., May 27, 1916, *P. Wilson 248;* West Orange, N. J., May 9, 1915, *P. Wilson 59;* Brownfield Woods, Urbana, Ill., May 18, 1919, *H. W. Anderson.*

TYPE LOCALITY: Madison, Wisconsin, on *Dicentra Cucullaria.*

II. Uredinia hypophyllous, few, scattered or in small groups, 1–2 mm. across, round, small, 0.1–0.2 mm., remaining partially covered by the epidermis, pulverulent, yellow, ruptured epidermis evident; paraphyses peripheral, hyphoid, 7–10 by 26–48 μ, thin-walled, colorless, incurved, inconspicuous, not projecting above the ruptured epidermis, arising from a more or less developed pseudoparenchymatous mass of mycelium; urediniospores ellipsoid or obovoid, 18–21 by 20–26 μ, without definite pedicels, attached to short, thin-walled, colorless cells; wall colorless, 1–1.5 μ thick, closely echinulate, the pores obscure.

III. Telia somewhat gregarious in groups 1–3 mm. across, at first arising within and surrounding the uredinia, angular, 0.2–0.5 mm. across, at first covered by the epidermis, becoming naked just before germination, waxy, slightly tinted, becoming flocculose and white from germination, teliospores cylindric or ellipsoid, 10–21 by 29–42 μ, catenulate, in chains of 2 or 3 at the center of the sorus, usually only one at the margin; wall colorless, very thin, uniformly 0.5 μ thick; basidiospores globoid, 10–13 μ in diameter.

Urticastrum divaricatum (L.) Kuntze (*Urtica divaricata* L., *Laportea canadensis* Gaud.), Brownfield's Woods, Urbana, Ill., Aug. 19, 1919, II & III, *H. W. Anderson;* Hannibal, Wis., July 27, 1920, III, *J. J. Davis.*

In the Urticastrum rust we have a telium differing from that of the

Nyssa rust principally in the catenulate method of spore formation, the teliospores developing in chains of two or three (figs. 4, 5). The terminal spore of these chains often germinates before the lower spores are fully developed, and it may be that more spores are produced from the sporogenous cell than show at any one time. The catenulate character of the teliospores indicates that this species is related to a group consisting of such genera as Phakopsora (Physopella, Bubakia, Schroeteriaster), Uredopeltis, Melampsoropsis, Chrysomyxa, Baeodromus, Alveolaria, and Cerotelium. In Alveolaria the arrangement of the teliospores in definite layers which separate from each other, and in Baeodromus the colored thick-walled teliospores with delayed germination, taken with the short life

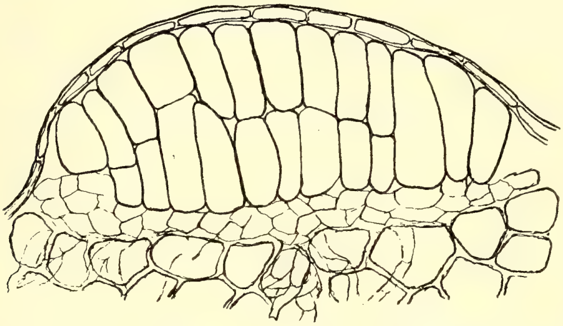


FIG. 4. Section through young telium of *Cerotelium Dicentrae* showing catenulate character of the teliospores.

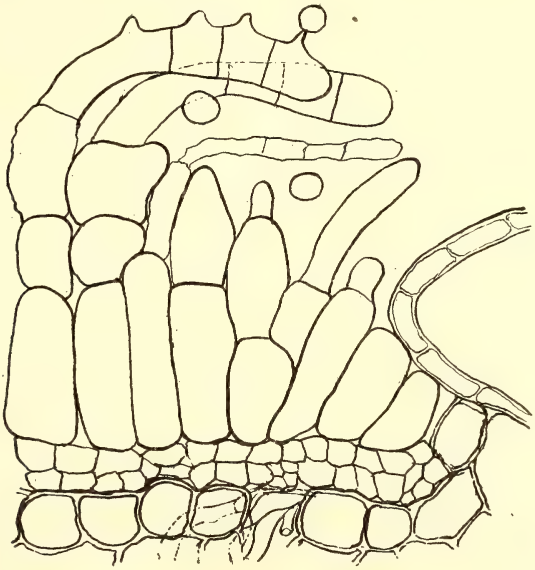


FIG. 5. Section through mature telium of *Cerotelium Dicentrae* showing germination of the teliospores.

cycle of both genera, afford reasons for excluding the Urticastrum rust from these genera. This rust is to be distinguished from species of Uredopeltis and Phakopsora by the colorless walls of the teliospores and by their germination without a resting period. Melampsoropsis, Chrysomyxa, and Cerotelium are genera in which the teliospores germinate without a resting period and are arranged in closely compacted chains. There is no characteristic of the telium which would necessarily prevent this rust from belonging to any of the last mentioned genera, except perhaps that there are a smaller number of teliospores in a chain in the Urticastrum rust than are usually found in the rusts of these genera.

The uredinium of the Urticastrum rust, however (fig. 6), is bordered by a few colorless, incurved paraphyses, and the walls of the echinulate urediniospores are colorless and the spores are borne in a manner similar to that in *Aplopsora Nyssae.* It therefore differs in these respects from species of Melampsoropsis (Chrysomyxa of some authors) which have catenulate, verrucose urediniospores, usually with a surrounding peridium. Chrysomyxa as used by Arthur lacks uredinia and in consequence will be disregarded. This species might be placed in the genus Cerotelium as originally described by Arthur[7] (p. 30), except for the presence of paraphyses in the uredinium instead of a peridium. As a result of the study of additional material Arthur[8] (pp. 505–507) brought together in each of the genera Phakopsora, Cerotelium, and Cronartium species with a peridium, species with paraphyses united below into a pseudoperidium, species with hyphoid or incurved paraphyses, and species with neither peridium nor paraphyses. In the first two genera, species can be found showing gradations, such as incurved colorless paraphyses, colored thick-walled paraphyses, paraphyses united to a greater or less extent to form a pseudoperidium, paraphyses accompanying a peridium, and a peridium only. In consequence the

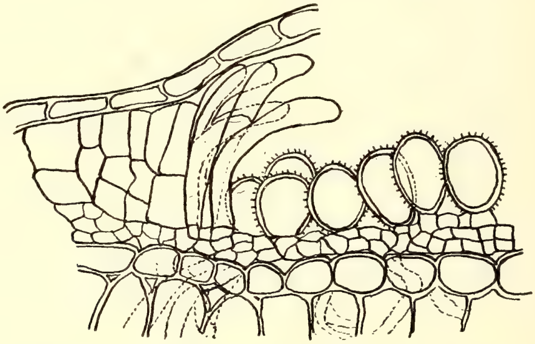


FIG. 6. Section through uredinium of *Cerotelium Dicentrae* showing peripheral paraphyses and manner in which urediniospores are borne.

---

[7] **Arthur, J. C.** New species of Uredineae. Bull. Torrey Bot. Club **33**: 27–34. 1906.

[8] **Arthur, J. C.** Relationship of the genus Kuehneola. Bull. Torrey Bot. Club **44**: 501–511. 1917.

presence of paraphyses seems no reason for excluding this rust from the genus Cerotelium, the smaller number of teliospores in chains being hardly more than is to be expected in a simpler species of the genus.

The Sydows[9] (pp. 524, 525) consider Cerotelium as a synonym of Dietelia and transfer the type species *Cerotelium Canavaliae* Arth. of the former genus to the latter genus. According, however, to their description of the genus Dietelia, the presence of a peridium around the telium is the most important characteristic of this genus. The telia of *C. Canavaliae* as such do not have a peridium, but when they arise in the old uredinial sorus, as they often do, they are of course surrounded by the peridium there present. Neither does the telial peridium of Dietelia resemble in structure the uredinial peridium of Cerotelium, and for these reasons the writer is of the opinion that Dietelia and Cerotelium should be considered as distinct genera.

In the discovery of the Aecidium of *Cerotelium Dicentrae* the first clue to an aecial stage of rusts of this type has been obtained. The combination of subcuticular pycnia with the cupulate aecium surrounded by a peridium is such as was to be expected from the relationship of the genus Cerotelium to other genera of the Melampsoraceae. The systemic nature of the mycelium is probably specific and accounts for the survival of *Cerotelium Dicentrae* in a temperate climate. The infection of *Bicuculla Cucullaria* by the basidiospores of the rust must occur through the dormant buds of the corm, which are either exposed or immediately below the surface of the soil. Such a type of infection would apparently necessitate a close association of the two alternate hosts and probably accounts for the rather localized occurrence of the rust.

Discussion of Relationships

The character of early maturity and germination of the teliospores has been given considerable prominence in establishing the position of these two rusts. This is a character which in some groups of the rusts is of little or no significance. Here, however, on account of the evident grouping and relationship of rusts with this character it appears to take on considerable importance. Thus if we consider the rusts of the Melampsoraceae which have teliospores germinating without a resting period, they would appear to group themselves in certain definite lines of development. Starting with Aplopsora we have teliospores in a one-layered crust which tardily breaks through the epidermis and germinates at once. In *Cerotelium Dicentrae* we have another step in which a number of the sporogenous hyphae cut off two and three spores in succession to form a compact crust which, on account of its continued spore formation, rather readily ruptures the epidermis. The next type is represented by *Cerotelium Canavaliae* in which the number of spores produced by the sporogenous hyphae is greater and in consequence the epidermis is quickly ruptured and the telium is

[9] **Sydow, P., and Sydow, H.** Monographia Uredinearum 3: 1–726. 1915.

pushed up farther above the epidermis. The telium here is less compact and with less evident lateral coherence of its spore chains. At this point in the development of this group, or a little before, a separation into two distinct lines apparently takes place. In one line there is an increase in the teliospore production from the sporogenous hyphae and a stronger lateral coherence of the spore chains; and hair-like columns of teliospores are formed, giving us the genus Cronartium. In the other line, there is also a greater spore production resulting in longer chains of teliospores, but at the same time the lateral coherence of the chains lessens until finally in the genus Kuehneola there is a complete separation to the base and a falling apart of the spore chains. In consequence of such a development, as might be expected, there is no sharp line of separation between the genera Cerotelium and Kuehneola and some species are in consequence difficult to place, some authors referring them to one genus and some to the other, depending upon their interpretation of the limitations of these genera. As an example of this transition from one genus to the other, we have the following: *Cerotelium Gossypii* (Lagerh.) Arth. possesses a compact telium much like that of *C. Canavaliae.* In *Cerotelium Fici* (Cast.) Arth. and in *C. Vitis* (Butl.) Arth., the teliospore columns are much more loosely arranged, as has been shown by Butler,[10] but the spore chains still hold together and show only a slight tendency to fall apart. In the case of *Kuehneola aliena* Syd. & Butl. and especially in *K. Butleri* Syd. we have two rusts which have been placed in the genus Cerotelium by Arthur (*l. c.* footnote 8, p. 510). In these species, although the spore chains are short and in consequence do not separate as widely as in some species of Kuehneola, the separation is, however, definite; and it would appear best to consider both as species of Kuehneola. Although Dietel[11] (pp. 205–213) was the first to point out the catenulate manner of teliospore formation in Kuehneola as distinguishing it from the genus Phragmidium, yet he retained the genus in the Pucciniaceae, considering it as having developed from Uromyces species on Rubus. Kuehneola was, however, removed to the family Melampsoraceae by Arthur (*l. c.* footnote 7), largely on account of this catenulate character of the teliospore, and these transitional forms support such a disposition of the genus.

The other genera of the Melampsoraceae with teliospores germinating without a resting period are Chnoopsora and Melampsoropsis. The former may be considered as arising from the same source as Aplopsora but diverging upon what may possibly be another line of teliospore formation. The latter may have arisen from a similar source, most likely from a form resembling Melampsora but differing in the development of catenulate urediniospores. The method of urediniospore formation in the Aplopsora-

---

[10] **Butler, E. J.** The rusts of wild vines in India. Ann. Mycol. **10**: 153–158. 1912. Especially pp. 156–158. Also, *l. c.* footnote 5, pp. 76–79.

[11] **Dietel, P.** Über die Verwandtschaftsbeziehungen der Rostpilzgattungen Kuehneola und Phragmidium. Ann. Mycol. **10**: 205–213. 1912.

Kuehneola line may possibly be considered as representing an original potentiality in the ancestral type which developed in Melampsoropsis but which in the Aplopsora-Kuehneola line became gradually weaker. Phakopsora with the delayed germination of its teliospores may be considered as a similar but less fully developed line from a source similar to that giving rise to Cerotelium.

Both the rusts described above have offered considerable difficulty in the determination of their generic position. It is in this, however, that their principal interest lies, for such species are to be expected as the result of evolutionary development and from such our knowledge of relationships must be obtained. With our present imperfect knowledge of the rusts of the family Melampsoraceae, it is perhaps impossible to gain more than a suggestion of the possibilities which exist. It is felt, however, that these two rusts, *Aplopsora Nyssae* and *Cerotelium Dicentrae*, in their evident relationships point to lines of development, the importance of which will have to be left for future information and studies fully to bring out.

To Prof. H. W. Anderson credit is due for the discovery of *Cerotelium Urticastri* and for the trouble he has taken in solving the life history of this rust. The writer wishes to express his appreciation to Dr. J. C. Arthur for the opportunity of studying the Urticastrum material and for advice and criticism in the study of these two interesting rusts. To Prof. H. S. Jackson acknowledgment is due for aid in obtaining additional material of *Aplopsora Nyssae* in the New York Botanical Garden herbaria and for helpful criticism of this work.

Purdue University Agricultural Experiment Station,
Lafayette, Indiana

MISCELLANEOUS STUDIES ON THE CROWN RUST OF OATS[1]

G. R. Hoerner

(Received for publication March 7, 1921)

During a somewhat extensive study of the infection capabilities of crown rust of oats, *Puccinia coronata* Cda. (1), the following data were collected.

I

McAlpine (2) ventures the opinion that crown rust of oats was probably introduced into Australia by means of seed. He does not state whether he thinks the rust was carried within or upon the seed in the form of mycelium or of urediniospores.

As far as surface-borne urediniospores are concerned, it seems questionable whether under ordinary conditions urediniospores would remain viable upon the seed surface long enough to be transported to any great distance and still be able, after relatively long periods of time and probably under adverse environment, to infect the developing seedlings. In an attempt to throw some light upon this question the following experiment was devised:

Twenty oat seeds of a variety known to be susceptible to crown rust (Victory, Minn. 514) were moistened in water and heavily smeared with fresh viable urediniospores. Five seeds were then planted about one half inch deep in each of four four-inch pots of a uniform soil mixture. These pots were placed in a ventilated cage in order to protect the developing seedlings from chance infection from air-borne spores.

After the seedlings had been allowed to grow for ten days, a sufficient period to show evidence of infection, it was found that none of the twenty seedlings became infected. The temperature within the cage was previously determined to be an optimum one, since artificial inoculations of seedling leaves of the same variety in the same environment resulted in normal infection, and moisture was present in sufficient amounts to cause guttation from the seedling leaves.

These results, though the experimental work was not extensive, would seem to indicate that in the case of *Puccinia coronata* Cda., urediniospores borne upon the surface of the seed do not commonly offer a favorable means of spreading the rust to the seedling plants developed from these seeds.

---

[1] Investigations carried on while the author was a graduate student at the University of Minnesota, 1916–1918.

Smithsonian Institution DEC 21 1921 National Museum

II

In the field, the soil beneath cereals heavily rusted with *P. graminis* Pers. is often found literally covered with fallen urediniospores. The idea has been conceived that seedlings penetrating such soil might become infected and the rust be aided in its spread in this way. Greenhouse experiments have proven this possible, it is reported, with *P. graminis* Pers. In order to determine if such infection is possible in the case of *P. coronata* Cda., the following experiment was devised:

After soaking in water for twenty-four hours, six oat seeds were planted about one half inch deep in each of four four-inch pots of a uniform soil mixture. The surface soil was then heavily dusted with fresh viable urediniospores. These pots were then placed in a ventilated cage to avoid possible chance infection of the seedlings by air-borne spores. Watering was avoided in order to prevent germination of the spores before the seedlings should come in contact with them. After ten days' time none of the twenty-two seedlings that developed showed any signs of infection, though guttation occurred from the seedling leaves, affording optimum conditions for spore germination.

These results indicate at least that seedling infection caused by emergence through soil densely covered with viable urediniospores does not occur readily. This condition may possibly be due to the fact that the sheath which surrounds the emerging seedling is not supplied with stomata and therefore affords no opportunity for the entrance of the germ tubes.

III

The possibility of urediniospore-producing mycelium overwintering in the host plant and producing a new crop of urediniospores in the spring, together with the overwintering of urediniospores in the field, was considered.

Christman (3) found, under Wisconsin conditions, viable urediniospores at any time during the winter with a three-months' period during which the temperature hovered about the freezing point. Urediniospores from oats, developed upon protected plants during the winter, germinated as late as January 26. Indications were that the mycelium would be as resistant as the host within which it grew. Old spores remained viable for some time, though new crops of spores from overwintered mycelium seemed to be the more important mode of spring infection.

Reed and Holmes (4) found viable urediniospores on oats throughout the year under Virginia conditions. They conclude that the crown rust on oats has an enduring mycelium capable of producing a new crop of spores during much of the winter, and although spore production ceases during midwinter, the mycelium, upon the advent of warm weather, is capable of producing new crops of viable spores.

The urediniospore-germination studies that the writer has performed would seem to indicate that under Minnesota conditions urediniospores cannot withstand the extremely low temperatures of winter. In the field, even before winter had set in, all urediniospores had disappeared and only the teliospores were in evidence. Two pots of heavily rusted oat plants were allowed to remain outside during the winter. The plants were winter-killed and when removed to the greenhouse in early April did not revive. The urediniospore-producing mycelium, if still alive, which one would naturally doubt, produced no new crop of spores.

From this more or less limited observational evidence, then, it seems improbable that under Minnesota conditions a perennial mycelium exists which is capable of producing a new crop of urediniospores the following spring after overwintering on the infected oat host, though Bolley and Pritchard (5) consider it in general quite possible, even though no experimental data are offered to substantiate the opinion. It seems equally improbable that the urediniospores themselves can overwinter and cause infection the following spring. Just what possibility there is of the existence of a perennial mycelium or of the overwintering of the urediniospores among the wild grasses, has not been determined.

IV

Mains (6), working with *Puccinia coronata* Cda., has shown that low temperatures, lack of moisture in the moist chamber, and the absence of light retard the development of the leaf rust of oats.

These same observations have been made in the present work, though only one definite experiment was performed and that to determine the effect of light upon the degree of infection and the rate of pustule formation.

Four pots of oats of the same seed lot, grown under the same conditions, were inoculated on the same date with inoculum from the same source. Two pots were placed in a pan of water and covered with a glass bell jar; the other two pots were given similar moisture and temperature conditions though covered by a glass-topped metal moist chamber from which light was excluded.

All four pots were removed from the moist chambers after forty-eight hours but retained for two days more under the light and dark covers. At the end of this period the plants in the dark had become spindly and distinctly yellowed. Flecks appeared on all the seedlings in all the pots at about the same time. Pustules ruptured within ten days upon the plants kept in the light and within twelve days on the plants kept in the dark. Infection, one hundred percent in each case, appeared normal on all the seedlings, though not so heavy on the plants grown in the dark. The plants that had been kept in the dark, after several days' exposure to the light showed nearly normal growth, though the effect of etiolation was evidenced by dead areas at the tips of the leaf blades.

Twenty-eight days after inoculation pigment appeared on one of the plants that had been exposed to the light, while twenty-six days after inoculation a profuse production of teliospores was noted on every plant in one of the pots that had been kept in the dark. (See figure 1, Plate XXIV.)

V

The appearance of a purple pigment surrounding infected areas of the oat leaves inoculated with crown rust is not uncommon. The variety of the host plant, its age before inoculation, the length of time of infection, the history of the inoculum, its method of application, and all other externally visible environmental factors seem to have no direct correlation with this phenomenon of pigment formation.

Wheldale (7), regarding this anthocyanin pigment formation in plants attacked by fungi, says:

> It is frequently found that the pathological conditions called forth by the attacks of fungi are accompanied by abnormal development of anthocyanin. In leaves of Tussilago, for instance, infected by Puccinia, a circular band of anthocyanin often appears surrounding the aecidium spots. . . . Injury to the living tissues of the conducting system of the veins, midrib or petiole of the leaf, or of corresponding tissues in the stem, leads to an accumulation of synthetic products in the leaves. . . . It seems likely also that parasitic growths may interfere with the progress of the translocation current through the small veins of the leaf, thereby causing congested areas to arise in which the sugar contents are above normal. But it is conceivable that the pathological condition resultant on fungal attacks may be the direct cause, in some way, of pigment formation.

In view of this interpretation and of the relatively general occurrence of this anthocyanin pigmentation during the course of the studies recorded in this paper, the assumption seems justified that pigment formation, as a phenomenon connected with the infection of oats by *P. coronata* Cda., is not a sign of resistance on the part of the host to the attacks of the rust parasite.

VI

Parker (8), speaking of early production of telia on oat seedlings, says:

> It is certain that in the hundreds of seedlings described as very susceptible in the present experiments telia were not produced on a single one following a normal and abundant production of uredinia.

In the investigations reported in this paper, this was not the case. Although certain oat varieties showing resistance to attacks of the crown rust did produce telia, other very susceptible varieties also produced telia freely and in great abundance. Super-susceptibility on the part of the host may bring about the formation of telia due to conditions as explained by Wheldale, although resistant hosts may react in some way so as to be unfavorable to continued urediniospore production on the part of the fungus, and thus hasten the completion of its life cycle and the early production of telia.

Parker has used this phenomenon of teliospore production, in certain cases, as a basis for the classification of resistant varieties. In view of results recorded here, such a basis for the classification of resistance would seem unreliable. Ligowa oats were listed as susceptible, and yet, during the course of these experiments, Ligowa oats, although heavily infected, produced pigment and telia both when growing under normal conditions and when subjected to adverse environmental circumstances. (See figure 1, Plate XXIV.) *Avena sterilis* L. is considered by Parker as for the most part susceptible. In the experiments here reported it produced pigment, telia, and extensive hypersensitive areas. Figure 2, Plate XXIV, shows leaves of *Avena sterilis* L. infected by *Puccinia coronata* Cda. The strain[2] of rust from Saint Paul, Minnesota, indicated by "S," caused a very light infection, the appearance of small scattered uredinia and large hypersensitive areas, and the early production of telia. The strain of rust from Tallulah, Louisiana, indicated by "T," caused heavy, normal infection without any evidence of pigment or telia formation. Swedish Select oats Parker considered susceptible, and yet in these experiments Swedish Select oats from Virginia produced telia in abundance. Appler oats Parker considered resistant. Figure 3, Plate XXIV, shows Appler oats from Alabama infected with *P. coronata* Cda. "T" shows normal infection with the strain of rust from Tallulah, Louisiana; "S" shows a heavy production of telia and extensive dead areas by the Saint Paul, Minnesota, strain. Parker considered Burt oats susceptible. In the present experiments Burt oats from Alabama showed a similar condition to that described for Appler.

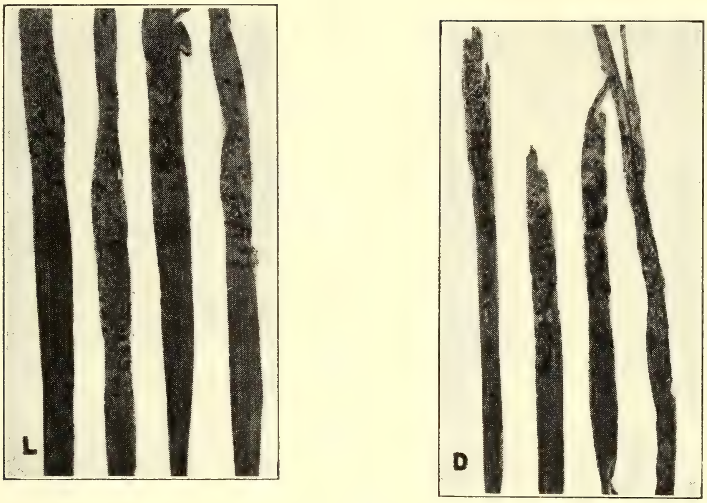
Therefore, though the production of telia when associated with other phenomena indicating resistance may be additional evidence to justify the classification of oat varieties as resistant, certainly results obtained in the present work seem to show that this phenomenon of telia formation on oat seedlings is variable and largely dependent upon environmental factors and possibly also upon the strain of rust employed, to such an extent at least as to make telia formation a rather unreliable basis for the determination of true resistance.

Summary

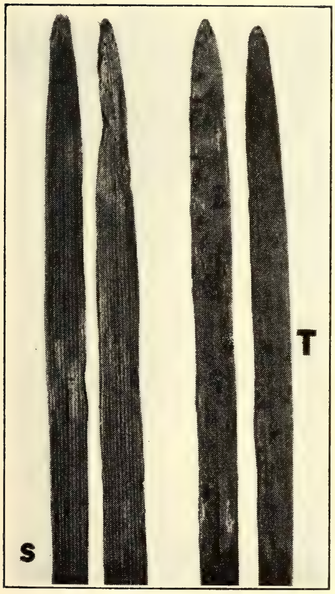
1. Urediniospores borne on the surface of oat seeds do not offer a ready means of infecting seedlings developed from these seeds.
2. Seedlings of oats emerging through soil heavily covered with viable urediniospores are not readily infected.
3. Under Minnesota conditions, a perennial mycelium, capable of producing a new crop of urediniospores after overwintering, does not exist. What the situation is in the case of wild grasses has not been determined.
4. Urediniospores do not remain viable over winter on oats, under Minnesota conditions, nor does continued production take place. What the situation is in regard to wild grasses has not been determined.
5. Environmental factors influence the development of the rust on oats as well as the rate of pustule formation.
6. Etiolation brings about the early formation of telia on oat seedlings.
7. Anthocyanin pigment formation surrounding uredinia on infected

---

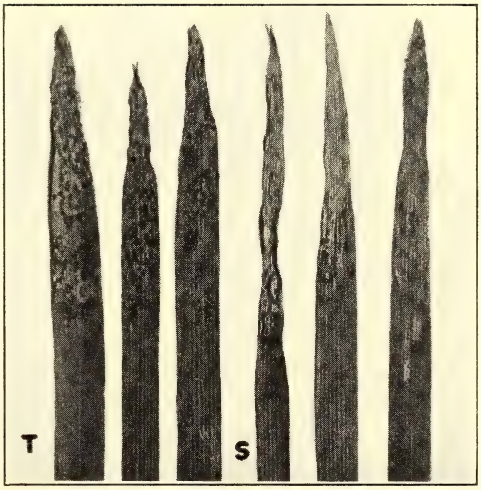
[2] The term "strain" is used to indicate merely a locality collection.



1



2



3

HOERNER: CROWN RUST OF OATS.

oat leaves is a common phenomenon though not correlated with resistance or susceptibility.

8. The appearance of telia on seedling oat leaves is not a reliable basis for determining the resistance of oat varieties.

Department of Botany and Plant Pathology,
Oregon Agricultural College,
Corvallis, Oregon

LITERATURE CITED

1. **Hoerner, G. R.** Biologic forms of *Puccinia coronata* on oats. Phytopath. **18**: 309–314. 1919.
2. **McAlpine, D.** The rusts of Australia. pp. 349. Melbourne, 1906.
3. **Christman, A. H.** Observations on the wintering of grain rusts. Trans. Wis. Acad. Sci., Arts, and Lett. **15**^{1}: 98–109. 1904.
4. **Reed, H. S., and Holmes, F. S.** A study of winter resistance of the uredospores of *Puccinia coronata*. Va. Agr. Exp. Sta. Ann. Rept. (**1911–12**): 18–21. 1913.
5. **Bolley, H. L., and Pritchard, F. J.** Rust problems. Facts, observations and theories. Possible means of control. N. Dak. Agr. Exp. Sta. Bull. **68**: 608–672. 1906.
6. **Mains, E. B.** The relation of some rusts to the physiology of their hosts. Amer. Jour. Bot. **4**: 179–220. 1917.
7. **Wheldale, M.** The anthocyanin pigments of plants. pp. 83. Cambridge, 1916.
8. **Parker, J. H.** Greenhouse experiments on the rust resistance of oat varieties. U. S. Dept. Agr. Bull. 629. 1918.

EXPLANATION OF PLATE XXIV

Fig. 1 (above). At "D" the teliospores formed on etiolated seedling oat leaves are shown; at "L" the normal production of urediniospores on the seedlings kept in the light.

Fig. 2 (below at left). *Avena sterilis* L. infected with *Puccinia coronata* Cda. "S" shows the production of telia and of extensive dead areas by the Saint Paul, Minnesota, strain of rust. "T" shows normal production of urediniospores by the Tallulah, Louisiana, strain.

Fig. 3 (below at right). Oats, Alabama-Appler 617, infected with *P. coronata* Cda. At "T" normal production of urediniospores by the Tallulah, Louisiana, strain of rust is shown; at "S," the heavy production of telia surrounded by hypersensitive areas produced by the Saint Paul, Minnesota, strain.

COMPARATIVE STUDIES ON RESPIRATION XVIII. RESPIRATION AND ANTAGONISM IN ELODEA

C. J. Lyon

(Received for publication March 19, 1921)

Previous studies in this series have dealt with the relation between antagonism and respiration, but have not included tissues of higher plants containing normal amounts of chlorophyll.[1] The experiments here presented were designed to test the effects of mixtures of solutions of sodium and calcium chlorides on such tissues.

For this purpose the leafy stems of *Elodea canadensis* were selected. This has proven to be excellent material since it is hardy in respect to climatic conditions and laboratory manipulation while sufficiently sensitive to reagents. The leaves are exceedingly thin, and gaseous exchange is very rapid.

All the plants were collected from one place in a slowly flowing stream and, as long as it remained open, taken fresh at least once a week to the laboratory. The material used during the late winter and early spring was provided by a quantity of plants collected in December and kept in the greenhouse in large glass jars in a cool room. It thrived well and when tested gave normal results.

The method used for most of the experiments was that developed by Haas[2] in which the plants were immersed in solutions containing an indicator.[3] The production of CO_2 was measured by the change in color of the indicator. The standard buffer solutions containing the same indicator were mixtures of borax and boric acid.

The final experiments were carried out by the use of the apparatus described by Osterhout.[4] The curves closely resemble those obtained by the other method. The accuracy of measurement was greater. Since the two methods are alike in all but mechanical details, only the first will be discussed in full.

The procedure consisted in measuring the normal rate of production of CO_2 in tap water or distilled water and then testing the effect of a salt solution on the same material in the same tube. For normal respiration the material was placed in 10 cc. of water to which had been added 5 drops of a 0.01 percent solution of phenolsulphonphthalein. Both tap and distilled water were tried, and as no difference could be noted in the effects on the plant, distilled water was used exclusively, since the salt solutions were made up in it. This eliminated possible effects of the salts in the tap water.

---

[1] The experiments on wheat reported in a previous paper of this series were made upon germinating seeds which contained little or no chlorophyll. Cf. **Thomas, H. S.** Jour. Gen. Physiol. 1: 203. 1918.

[2] **Haas, A. R. C.** Science N. S. 44: 105. 1916.

[3] The plants were thoroughly washed to remove any adhering organisms. Microscopical inspection showed that the plants used for the experiments were almost free from bacteria.

[4] **Osterhout, W. J. V.** Jour. Gen. Physiol. 1: 17–22. 1918.

Both the water and the salt solutions were brought to the proper alkalinity by the addition of a very dilute solution of sodium hydroxide, the same amount being added to each.

The plants selected were healthy stems, uniform in appearance, which averaged from 3 to 4 inches in length. These were kept in running water before use (to remove any excess of CO_2), and were then coiled and inserted in the tubes where the pressure of the coil held them in the middle of the tube. This kept them from interfering with the observation of the color of the solution (in the lower half of the tube) and with comparison with the color of a standard solution. The paraffined rubber tube at the top was then tightly clamped off after adding the water plus the indicator. A bubble of air, of uniform size in all experiments, was left below the clamp to aid in stirring the solution. The pH value of the water was brought to a little above 7.88, but it dropped to 7.88 shortly after the plants were placed in it. The exact time at which this point was reached was determined by matching its color with that of a buffer solution of pH 7.88 (which had the same concentration of indicator).

The contents of the tube were kept in constant motion by gentle stirring during the few minutes required for the evolution of enough CO_2 by the plant to change the color to match that of the second standard tube of pH 7.60.[5] This range of 7.88 to 7.60 was used in all experiments. In getting the normal rate, the amount of material used was adjusted to give a period of from 3 to 5 minutes in most cases.

That no acid other than carbonic was produced was shown by the fact that after the plant had changed the color of the indicator solution, it would rapidly return to the original color when a current of CO_2-free air was bubbled through it.

The normal period of respiration for each experiment was first determined. It was usually found that this period was practically constant for two hours or more, and if this was not the case the material was rejected. At least three readings (covering a period of at least 20 minutes) were taken, previous to the addition of the salt solutions, in order to establish the normal rate.

The temperature varied from 21 to 25° C. In the course of any one experiment, the temperature did not vary more than two degrees.

The solutions of salts were made up in large quantities and kept in bottles of resistant glass. It was necessary to have them well stoppered, and to keep the bottles well filled in order to avoid absorption of carbon dioxide and the consequent reduction of alkalinity and increase of buffer action when made alkaline again.

---

[5] The source of light was a "Daylight lamp." Cf. **Luckiesch.** Science n. ser. **42**: 764. 1915.

Preliminary experiments showed that a concentration of 0.1 M sodium chloride was preferable for study. Anything above 0.2 M was found to give plasmolysis. A solution of 0.07 M of calcium chloride was taken as approximately isotonic with the 0.1 M sodium chloride, and all mixtures were made up with these concentrations.

In order to avoid a possible error by the buffer action of the solutions, these were tested by bringing the distilled water and all the solutions to an alkalinity of pH 8 and then adding a few drops of a solution of CO_2 in distilled water. All changed by approximately the same amount, which showed that there was no appreciable buffer action that would interfere in the measurements of production of CO_2.

The time curves of the production of CO_2 were plotted in the manner explained by Osterhout[6] and used in other papers in this series. Rate of respiration in percent is plotted against time in minutes, the normal rate (as determined before addition of salt) being taken as the reciprocal of the average period required to change the solution from PH 7.88 to 7.60; this rate was taken as 100 percent.

The behavior of the plant was not quite the same in the fall and spring seasons. In early spring, while working with material kept in the greenhouse, it was found that the solutions of pure salts and the mixtures were giving different rates of production of CO_2 than the same solutions had in the fall and winter. The shapes of the curves were not changed, but there was an increase in all the ordinates of from 5 to 15 percent. This prevented the inclusion of many data, for there were not enough experiments for certain points to give a complete curve by themselves. Nevertheless, they provide confirmation of the results presented; especially of the maximum points in figure 2.

Figure 1 shows the production of CO_2 in NaCl 0.1 M (curve A), in $CaCl_2$ 0.07 M (curve B), and in mixtures of these. It was found that all concentrations of NaCl (none above 0.2 M were tried) gave an increase,[7] while all those of $CaCl_2$ gave a decrease. In some of the weaker concentrations of $CaCl_2$ the rate showed a tendency to rise again after falling, but it remained below the normal. In low concentrations of NaCl the increase (of from 25 to 50 percent) lasted for at least 90 minutes, while in 0.1 M NaCl it fell off rapidly at first and then more slowly as shown in the typical curve.

A remarkable behavior was observed when the molecular proportions were 98.62 NaCl to 1.38 $CaCl_2$; the rate increased and did not return to normal (as shown in curve D, figure 1).

---

[6] **Osterhout, W. J. V.** Jour. Gen. Physiol. 1: 171–179. 1918.

[7] B. Jacobi (Flora **86**: 289. 1889) states that NaCl 0.0496 M causes an increase in the production of CO_2 by Elodea, followed by a decrease.

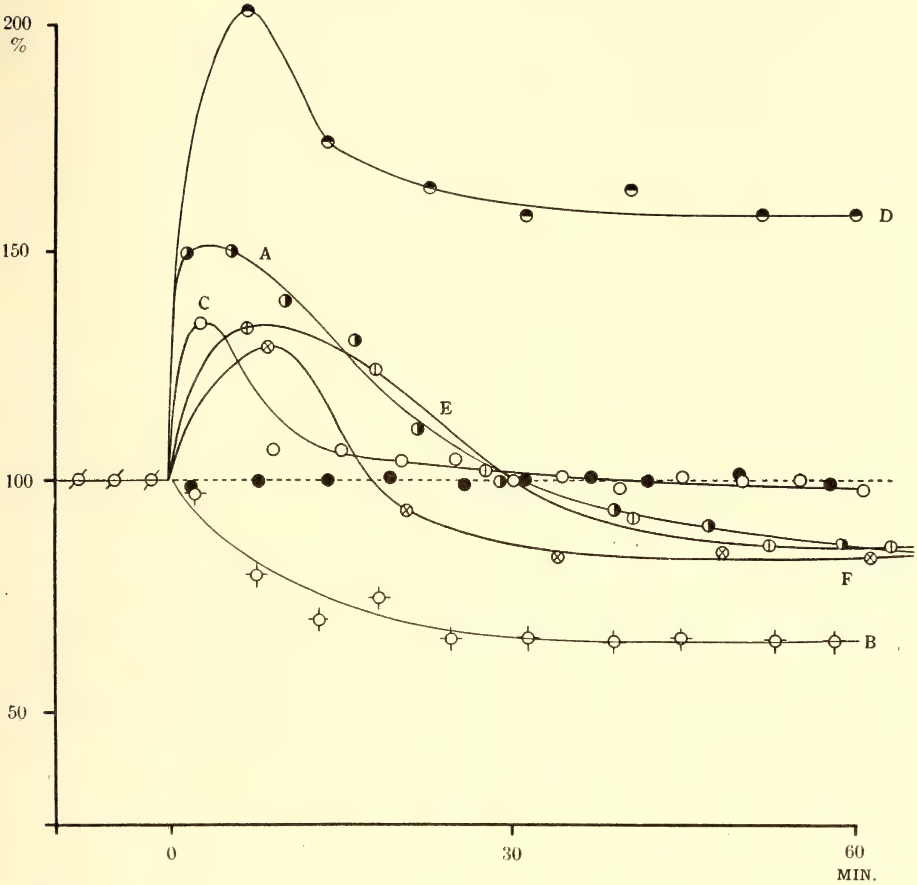


FIG. 1. Curves showing the effects of NaCl and $CaCl_2$ on the respiration of *Elodea canadensis.* The horizontal line at the left of the point marked O on the abscissae represents the normal rate of respiration before the addition of the salt.

Curve A represents the rate of respiration in NaCl 0.1 M, curve B in $CaCl_2$ 0.07 M; the other curves represent the rate in mixtures of these having the following molecular percentages: curve C in 99.65 percent NaCl+0.35 percent $CaCl_2$; curve D in 98.62 NaCl +1.38 $CaCl_2$; curve E in 98.85 NaCl+1.15 $CaCl_2$; curve F in 98.28 NaCl+1.72 $CaCl_2$. The broken line represents the control in distilled water. Each curve represents a typical experiment.

The determination of antagonism should preferably be made at a period of the experiment when both solutions of pure salts give either an increase or a decrease in rate. Since the former was impossible, an exposure of one hour was chosen, at which period both gave a decrease and the time curves had become nearly horizontal. Figure 2 shows the rates of respiration in various mixtures and in the solutions of pure salts. The ordinates represent

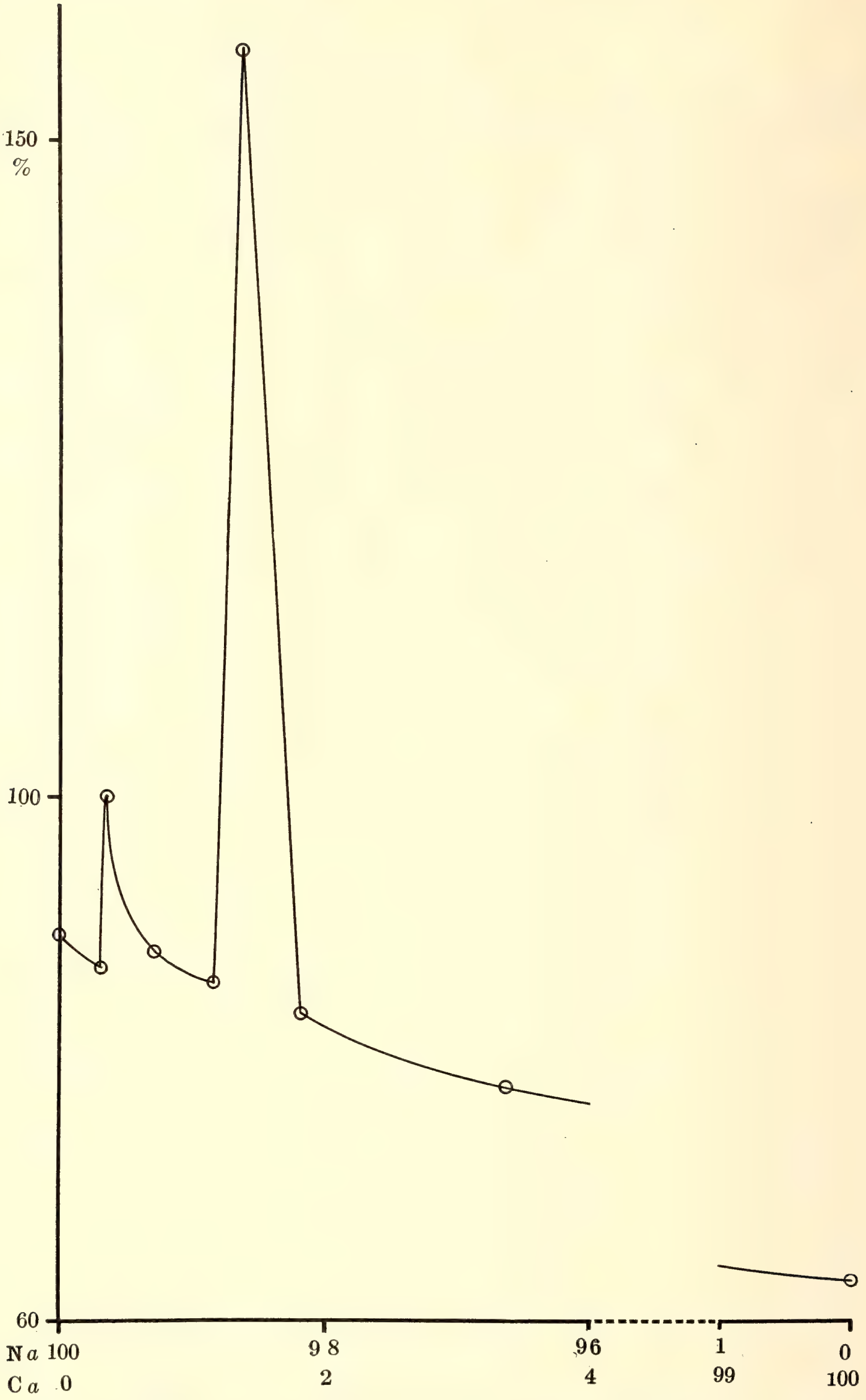


FIG. 2. Antagonism curve showing the effect of NaCl 0.1 M+CaCl$_2$ 0.07 M and of mixtures of these upon the respiration of *Elodea canadensis* after an exposure of one hour. The abscissae represent molecular proportions. Each point represents the average of 3 or more experiments; probable error of the mean, less than 4 percent of the mean (except in one case where it amounts to less than 6 percent).

the rate of production of CO_2 after the plants had been in the solutions for one hour.

The figure shows that while the molecular proportions 99.65 NaCl to 0.35 of $CaCl_2$ give normal respiration, a decrease occurs at other proportions except that of 98.62 to 1.38 of $CaCl_2$. This form of the antagonism curve of NaCl vs. $CaCl_2$ is unique, as is evident on comparing it with others in this series as well as with those in which growth, length of life, etc., are used as criteria.[8] In order to explain this peculiar effect additional experiments will be necessary, and further discussion is deferred until these can be carried out.

SUMMARY

1. Solutions of NaCl cause an increase in respiration, which is followed by a decrease, while solutions of $CaCl_2$ cause only a decrease.

2. After a sufficient length of exposure both NaCl and $CaCl_2$ depress the respiration. In a mixture containing 99.65 mols of NaCl to 0.35 of $CaCl_2$ the rate remains normal, while a mixture of 98.62 mols of NaCl to 1.38 of $CaCl_2$ causes a great increase in respiration.

3. The antagonism curve of NaCl vs. $CaCl_2$ is unique in that it has two maxima.

LABORATORY OF PLANT PHYSIOLOGY,
HARVARD UNIVERSITY

---

[8] Antagonism curves with two maxima have been reported for other combinations of salts, but they seem to be somewhat different in character. Cf. **Osterhout, W. J. V.** Bot. Gaz. **48**: 98. 1909. **Brooks, M. M.** Jour. Gen. Physiol. **2**: 5. 1919. **Lipman, C. B.** Bot. Gaz. **48**: 105. 1909; Bot. Gaz. **49**: 41. 1910. **Loeb, J.** Jour. Biol. Chem. **28**: 175. 1916.

THE EFFECT UPON PERMEABILITY OF (I) THE SAME SUBSTANCE AS CATION AND ANION, AND (II) CHANGING THE VALENCY OF THE SAME ION

Oran L. Raber

(Received for publication April 6, 1921)

I. The Effect of the Same Substance as Cation and Anion

In a recent paper (1) facts were presented which indicate that polyvalent cations do not cause an increase in the resistance of Laminaria if they are combined with polyvalent anions, and it seems natural to suppose that the difference between the action of a salt with a bivalent cation and a monovalent anion, *e.g.*, $MgCl_2$, and one with a bivalent cation and a bivalent anion, such as $MgSO_4$, is due to the extra charge on the anion. Cations seem to cause an increase in resistance and anions a decrease. In $MgCl_2$ the action of the cation is dominant; in $MgSO_4$, the action of the anion.

If this effect is due to electrical charge, we may ask what will happen if the same ion is used as cation and as anion. This problem seems at first glance to present great difficulties because it is necessary to work with solutions which are not strongly acid or alkaline. Certain elements (such as aluminum, arsenic, chromium, etc.) exist both as cations and as anions, but they are very weak acids or bases and solutions of their salts are not neutral.

The difficulty may be surmounted in a measure, however, by adding enough acid to the alkaline solution to bring its pH value down to that of the acid solution. If necessary the conductivity may be increased by mixing with a neutral salt (*e.g.*, NaCl).

For this purpose, chromous chloride, sodium chromate, chromic acid, and sodium chloride were used. The chromous chloride is not soluble enough to make the conductivity equivalent to that of normal sea water, and in order that the osmotic pressure of the sea water (with which the solution is compared) should not be too low, the chromous chloride was mixed with sodium chloride. The final solution was then composed of 50 percent chromous chloride 0.61 M and 50 percent sodium chloride 0.52 M. This mixture has greater electrical resistance than sea water and has a pH of about 4.5 as determined by the hydrogen electrode.

When tissue is transferred to this mixture from diluted sea water of the same conductivity, the following changes in resistance are observed (see fig. 1, *A*):

Time in Minutes	Percentage of Original Resistance	Probable Error of the Mean Expressed as Percentage of the Mean (Three Experiments)
2	101	0%
5	102	1
10	92	1
20	75	6
40	51	8
60	47	11
80	46	11

It is seen that there is a slight rise of resistance which is quickly followed by a fall. That the rise is no greater may be due to the facts that (1) the chromium ion used carries only two charges, and (2) the dilution with the sodium chloride would also tend to cause an immediate fall in resistance.

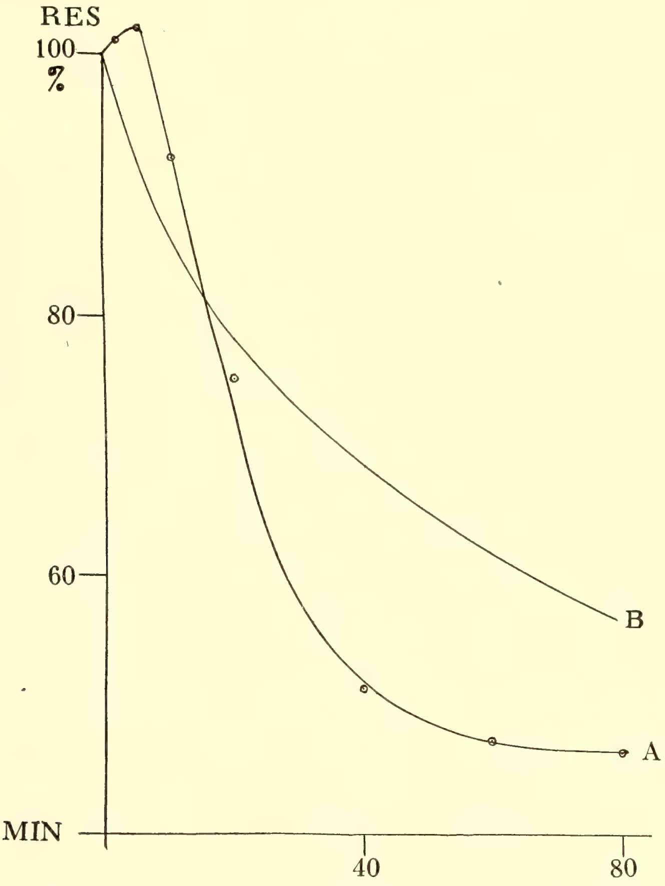


FIG. 1. *A*. Effect of 50 percent $CrCl_2$ 0.61 M plus about 50 percent NaCl 0.52 M upon the permeability of *Laminaria Agardhii* Kjellm. Ordinates indicate percentage of the original resistance (considered as 100 percent) in sea water of the same conductivity as the solution tested. Abscissae represent time in minutes of the tissue in the solution. *B*. Effect of a solution of 50 percent Na_2CrO_4 0.22 M and 50 percent NaCl 0.52 M with enough chromic acid added to produce the same pH as in *A* (pH 4.5).

Sodium chromate of 0.22 M has about the same conductivity as normal sea water and has a pH of about 9.5. This is not alkaline enough to cause any appreciable effect, just as a pH of 4.5 was not acid enough to interfere with the success of these experiments, as shown by Osterhout (2). Nevertheless, in order that conditions might be comparable with those in which chromium was used as a cation, a solution was prepared consisting of 50 percent sodium chromate 0.22 M, 50 percent sodium chloride 0.52 M, and with just enough chromic acid added to bring the acidity up to that of the chromous chloride solution (pH about 4.5).

The acidity in the case of colored solutions was measured by diluting a hundred times, taking the pH of this diluted solution, and then making a correction for the increase in dissociation upon dilution. As a check upon this method the pH was also determined directly by means of the hydrogen electrode.

The results obtained with the sodium chromate mixture of pH 4.5 are shown below and in figure 1, *B*. The figures given are the average of three experiments.

Time in Minutes	Percentage of Original Resistance	Probable Error
2	93	1%
5	88	1
10	85	2
20	78	2
40	68	3
60	62	3
80	55	5

It is seen that the initial effect is a fall in resistance. This is to be expected since we have here a monovalent cation with a bivalent anion. It is also seen that the hydrogen-ion concentration is not responsible for the rise in resistance when the chromium has a positive charge. When it has a negative charge (even though the hydrogen-ion concentration is the same, *viz.*, pH 4.5) the resistance does not increase but, on the contrary, decreases from the start. To what extent this is due to the presence of the oxygen in the anion can not be determined.

II. The Effect of Changing the Valency of the Same Ion

As previously stated, the nature of the charge on the ion seems to be a very important factor in determining the initial response of the tissue to electrolytes. It is natural to inquire what the result would be if the same ion could be used with a varying charge. If an anion with one charge causes a decrease in resistance, the same ion with two or three charges should cause a more rapid decrease. Similarly, a cation with two positive charges should cause a less rise in resistance than one with three provided they are used with anions which permit this rise in resistance. Osterhout found in general a greater increase in resistance with trivalent and tetravalent cations (3) than with bivalent cations (4), but he did not compare the effects of the same ion with different charges.

The choice of ions for this purpose is very limited. Antimony, arsenic, and tin can not be used because of their weak basicity which results in extremely acid solutions. The toxic action of copper is a sufficient reason for not using it. The insolubility of mercurous, chromic, and cobaltic salts almost prohibits their use. This leaves iron as a possibility among the common cations of variable valency.

For this study $FeCl_3$ and $FeCl_2$ were used. The solution of $FeCl_3$ was 0.20 M and had a pH value of about 2.5. When the tissue was placed in such a solution having the same conductivity as normal sea water, there occurred a very sudden and temporary rise in resistance followed by a rapid fall. The following table and figure 2, *A* show the results:

Time in Minutes	Percentage of Original Resistance	Probable Error (Three Experiments)
2	125	1%
5	107	1
10	71	5
20	55	9
40	47	8
60	44	7
80	42	7

The tissue becomes extremely hard and decidedly yellow in color, which seems to be due to the acid reaction of the solution. Inasmuch as the acidity of the ferric chloride lies outside the ineffective limit (2), a certain amount of the rise in resistance shown above is doubtless due to the hydrogen ions present.

The $FeCl_2$ solution of the same conductivity as that of $FeCl_3$ used is about 0.28 M and has a pH of about 4, but in order to compare the effect of the valency the solution was acidified by the addition of HCl. The acidity of these solutions (since colored ions are present) is measured and compared in the same way as that of the solutions discussed in the previous section. The final solution used consisted of 45 g. $FeCl_2$ per liter with enough HCl added to make a solution of the same conductivity and the same pH as the solution of $FeCl_3$. The results of this set of three experiments are given below and in figure 2, *B*.

Time in Minutes	Percentage of Original Resistance	Probable Error
2	118	4%
5	91	5
10	68	5
20	46	4
40	31	6
60	28	3
80	27	3

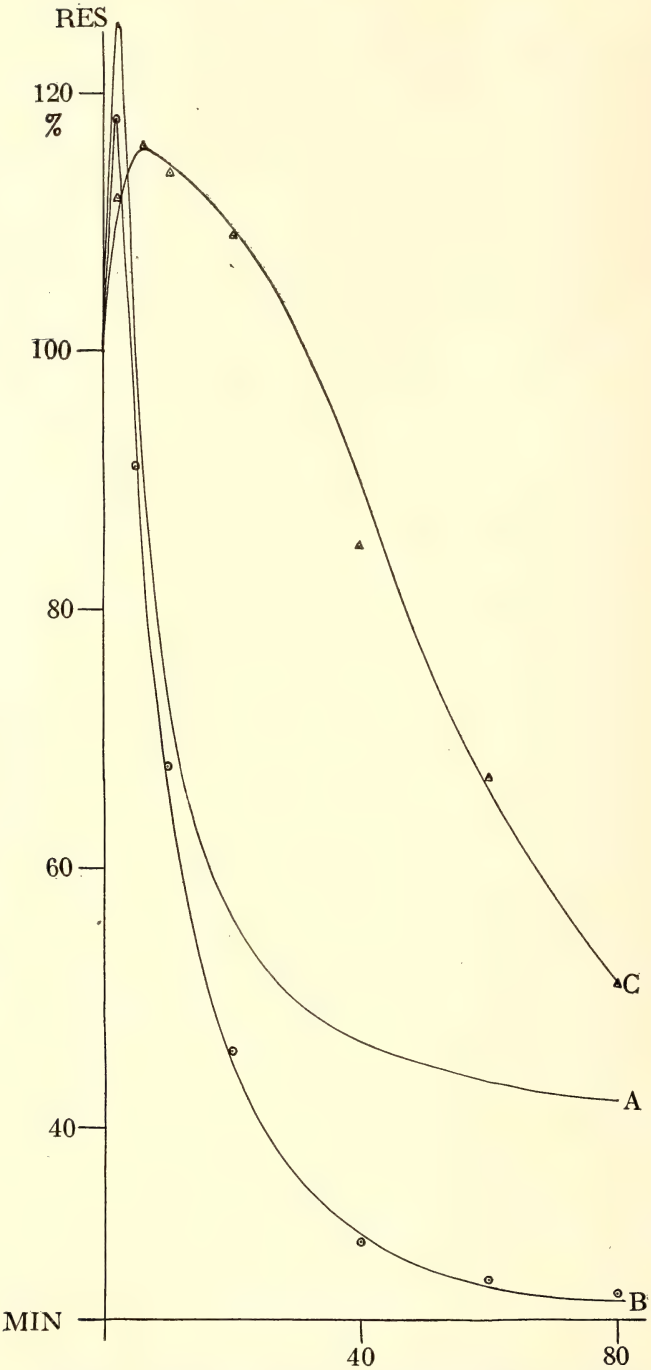


FIG. 2. *A*. Effect of $FeCl_3$ 0.20 M upon the permeability of *Laminaria Agardhii* Kjellm. Ordinates indicate percentage of the original resistance (considered as 100 percent) in sea water of the same conductivity as the solution tested. Abscissae represent time in minutes of the tissue in the solution. *B*. Effect of $FeCl_2$ 0.23 M with enough HCl added to produce the same hydrogen-ion concentration as in *A* (pH 2.5). *C*. Effect of $FeCl_2$ 0.28 M of the same conductivity as *A* and *B* (pH 4).

It is to be noted that the two curves are similar. The ferrous curve lies a little below the ferric curve. Even though the two are of the same acidity, the ferric causes a greater rise in resistance at the start than the ferrous solution. The additional positive charge on the cation seems to be the chief cause of the difference between these two solutions.

In order to see how much of the action of these salts is due to the high acidity, a third set of experiments was performed using 0.28 M $FeCl_2$ of the original pH (about 4). This concentration is the equivalent of a 0.0001 N HCl solution and, as mentioned above (2), this degree of acidity has been found to have no appreciable effect upon the permeability of Laminaria. The results of these three experiments are shown below and in figure 2, *C*.

Time in Minutes	Percentage of Original Resistance	Probable Error of the Mean
2	112	1%
5	116	2
10	114	4
20	109	3
40	85	4
60	67	7
80	51	10
100	40	10

The tissue here retains its original color and (as shown by the table and figure) the increase in resistance takes more than twice as long to reach a maximum. The general behavior and appearance of the tissue is very different from that in the two previous cases, and has in common with them only the initial increase followed by a decrease in resistance. The greater rise in the ferric chloride is not due to the concentration, since the ferric chloride is really less concentrated than the ferrous, the former being 0.20 M and the latter 0.23 M.

The conclusion would seem to be that in the case of curves *A* and *B* the primary factors are the ferric, ferrous, and chlorine ions while the hydrogen ion plays a subordinate role. The acidity causes the maximum rise to be arrived at much earlier and causes the extremely rapid fall. The valency of the cation, however, determines largely the height to which the resistance rises.

Summary

1. Chromium has a different initial effect upon the permeability of Laminaria depending upon whether it occurs in the cation or in the anion of a salt. If it is in the anion, the first effect is a decrease in resistance, and if in the cation, an increase.

2. Ferric chloride causes a greater increase in resistance than ferrous

chloride, independent of the hydrogen-ion concentration. The difference seems to depend upon the valency.

LABORATORY OF PLANT PHYSIOLOGY,
HARVARD UNIVERSITY

LITERATURE CITED

1. **Raber, O. L.** The effect upon permeability of polyvalent cations in combination with polyvalent anions. Amer. Jour. Bot. **8**: 382–385. 1921.
2. **Osterhout, W. J. V.** The effect of alkali on permeability. Jour. Biol. Chem. **19**: 335. 1914. The effect of acid on permeability. Ibid. **19**: 493. 1914.
3. ——. The effect of some trivalent and tetravalent cations on permeability. Bot. Gaz. **59**: 464. 1915.
4. ——. On the decrease of permeability due to certain bivalent cations. Bot. Gaz. **59**: 317. 1915.

BOTANICAL SOCIETY OF AMERICA

Reorganized 1906, uniting the Botanical Society of America (1893), the Society for Plant Morphology and Physiology (1896), and the American Mycological Society (1903).

OFFICERS FOR 1921

President: C. E. Allen
University of Wisconsin

Vice-President: H. C. Cowles
University of Chicago

Secretary: J. R. Schramm
Cornell University, Ithaca, N. Y.

Treasurer: Irving W. Bailey
Bussey Institution, Harvard University

Councilors:

N. L. Britton
New York Botanical Garden

William Trelease
University of Illinois

J. C. Arthur
Purdue University

Any one actively interested in botanical work is eligible for membership. Candidates for membership are recommended by three members, not members of the Council, on blanks to be obtained from the Secretary.

The annual dues are $5.00, including subscription to the American Journal of Botany. Candidates proposed for membership, and approved by the Council, may receive one copy of the Journal at $4.00 per year pending their election at the winter meeting of the Society.

The Little Things That Count Big In Owning Your Own Greenhouse

Ever gone into a hat store, knowing exactly the kind and shaped hat you want; and then have some smart Alec of a salesman try to sell you the kind HE likes?

Makes a fellow madder than a wet hen, doesn't it?

Suppose, however, that same salesman had told you something about some of his hats that meant better service to you—more satisfaction for your money—you then sort of feel towards that salesman as you would an old friend.

Salesman number two is our sort when it comes to selling a greenhouse.

We do sell greenhouses, but we first sell satisfaction.

Catalog for the asking.

Hitchings and Company

Home Office and Factory: ELIZABETH, N. J.

NEW YORK
101 Park Ave.

BOSTON-9
294 Washington St.

Vol. VIII. DECEMBER, 1921 No. 10

AMERICAN JOURNAL OF BOTANY

OFFICIAL PUBLICATION OF THE
BOTANICAL SOCIETY OF AMERICA

CONTENTS

Pollen and pollen enzymes . . . . JULIA BAYLES PATON 471

The embryogeny of Cyrtanthus parviflorus Baker . . WM. RANDOLPH TAYLOR 502

Studies on plant cancers III. The nature of the soil as a determining factor in the health of the beet, Beta vulgaris, and its relation to the size and weight of the crown gall produced by inoculation with Bacterium tumefaciens . . . . MICHAEL LEVINE 507

Index to Volume VIII 526

Smithsonian Institution
FEB 18 1922
National Museum.

PUBLISHED
IN COOPERATION WITH THE BOTANICAL SOCIETY OF AMERICA
BY THE
BROOKLYN BOTANIC GARDEN
AT 41 NORTH QUEEN STREET, LANCASTER, PA.

Entered as second-class matter February 21, 1914, at the post office at Lancaster, Pennsylvania, under the act of March 3, 1879.

AMERICAN JOURNAL OF BOTANY

DEVOTED TO ALL BRANCHES OF BOTANICAL SCIENCE

ESTABLISHED 1914

EDITED BY A COMMITTEE OF THE
BOTANICAL SOCIETY OF AMERICA

EDITORIAL COMMITTEE

C. E. ALLEN, Editor-in-Chief,
University of Wisconsin

WILLIAM CROCKER,
The Thompson Institute For Plant Research, Inc.

L. R. JONES,
University of Wisconsin

C. STUART GAGER, Business Manager
Brooklyn Botanic Garden

ARTHUR H. GRAVES
Brooklyn Botanic Garden

R. A. HARPER,
Columbia University

JACOB R. SCHRAMM,
Cornell University

C. L. SHEAR, *Bureau of Plant Industry*
(Representing American Phytopathological Society)

The Journal is published monthly, except during August and September. Subscription price, $6.00 a year. Single copies 75 cents. Back numbers, 75 cents each; $7.00 a volume. Postage will be charged to all foreign countries, except Mexico, Cuba, Porto Rico, Panama Canal Zone, Republic of Panama, Hawaii, Philippine Islands, Guam, Samoan Islands, and Shanghai. Postage to Canada, 20 cents a volume on annual subscriptions; to all other countries in the Postal Union, 40 cents a volume on annual subscriptions.

The pages of the Journal are open to members of the Botanical Society of America, or to candidates approved for membership.

Manuscript offered for publication should be typewritten, and should in all cases be submitted to the Editor-in-Chief.

Papers are limited to 20 pages in length, exclusive of plates, except that additional pages may be arranged for at cost rate to authors (approximately $3.00 a page).

Proofs should be corrected immediately on receipt, and returned to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y.

Illustrations, other than zinc etchings, and tabular matter should, in general, be restricted to approximately ten percent of the number of pages of text. Authors may be charged at cost rates for illustrations and tabular matter in excess of that amount (approximately $6.40 a page for tabular matter; 15 cents per square inch for half-tones).

Separates should be ordered when proof is returned. Ffty copies without cover will be supplied free; cover and additional copies at cost.

Remittances should be made payable to American Journal of Botany. Ten cents must be added to all checks not drawn on New York City banks.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

Correspondence concerning editorial matters should be addressed to Prof. C. E. Allen, University of Wisconsin, Madison, Wisconsin.

Business correspondence, including notice of change of address, and directions concerning reprints, should be addressed to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y., or 41 North Queen Street, Lancaster, Pa.

AMERICAN
JOURNAL OF BOTANY

VOL. VIII DECEMBER, 1921 No. 10

POLLEN AND POLLEN ENZYMES

JULIA BAYLES PATON

(Received for publication March 28, 1921)

I. THE THEORETICAL AND PRACTICAL ASPECTS OF THE OCCURRENCE OF POLLEN ENZYMES

Reasons for Undertaking the Investigation

A review of the literature shows very few complete or satisfactory reports of experiments in regard to either the general chemistry or the enzymes of pollen. Our knowledge of the subject seems to be very fragmentary. It is conspicuous by its omission from the textbooks of botany. Aside from the few references given later, up to the present time no mention of any important work has been found.

Although it is generally assumed, and is stated in our textbooks, that the pollen tube digests its way through the tissues of the pistil and the ovule, yet there seems to be no experimental evidence as to the exact nature of this enzyme action. Besides this, pollen enzymes must be very important in rendering the food stored in the grain available when the pollen germinates, in nourishing the tube during its passage through the style, and in stimulating the development of the embryo and the maturing of the ovary.

Moreover, pollen anaphylaxis is now regarded as the cause of so-called hay fever and other forms of pollen poisoning. Pollen enzymes may be concerned in these reactions, and the proteolytic enzymes may affect the stability of the pollen-protein solutions used in pollen vaccination.

In view, therefore, of the apparent meagerness of our knowledge of pollen enzymes and of the possible practical value of any contribution to this subject, it has seemed worth while to study the matter and to present the results.

The Literature of Pollen Enzymes

Few original, systematic experiments have been reported. Erlenmeyer (1874) found amylase, or diastase, in pine pollen. Van Tieghem (1869) reported invertase, or invertin, in the pollen of hyacinth, narcissus, wallflower, and violet. Czapek (1905, p. 393) quotes Strasburger's statement

[The Journal for November (8: 425-470) was issued December 19, 1921.]

that the pollen tubes of *Agrostemma Githago* bore through the membrane of the stigma papillae as evidence for a cytase in pollen. Czapek also refers to the investigations of Rittinghaus (1886, pp. 105–122) as confirming the opinion of Strasburger. The observations of Rittinghaus may, however, be interpreted quite differently, and point quite as definitely to the presence of a pectinase, as of a cytase. Rittinghaus examined numerous flowers, including Ipomoea, Convolvulus, Alisma, Agrostemma, Lychnis, Phlox, and Silene. He writes (p. 111):

> Die Verschmelzung zwischen der Cuticula der Papille und der Cellulosemembran des Schlauches ist ganz deutlich zu erkennen, und es leuchtet ein, dass die Lücke in der Cuticula ihre Entstehung nur einer unmittelbaren Einwirkung der Pollenschlauchspitze verdankt. Das lösende Agens ist somit nur im Plasma des Pollenschlauches zu suchen. Über die Natur desselben ist einstweilen leider nichts zu eruiren, zumal das einzige uns bekannte Cuticula-lösende Reagens kochende Kalilauge ist. Vielleicht wird man später die Erscheinung durch die Gegenwart eines besonderen Enzymes aufklären können.

J. R. Green (1891) noted amylase in pollen tubes. Green's later researches in 1894 are by far the most careful and complete experiments on pollen enzymes which have so far been reported. They will be briefly reviewed on a later page. Strasburger (1886) mentions diastase and invertin as present in pollen grains prior to germination. Sandsten (1909) reports invertase and diastase. Later, Kammann (1904) found protease, diastase, catalase, and lipase in rye pollen but does not give details of his experiments.

In the investigations of Green (1894, pp. 385–409) the pollen was powdered with glass and the powder suspended either in glycerine, or in a 5 percent solution of NaCl, to which 2 percent of potassium cyanide was added as an antiseptic. In other cases chloroform (a few drops) or oil of cinnamon was used as an antiseptic. The 5 percent NaCl solution proved preferable to glycerine. Diastase was found in the pollen of Gladiolus, Anemone, Antirrhinum, Tropaeolum, Pelargonium, Crocus, Brownea, Helleborus, Alnus, Tulipa, and Clivia; also in that of Zamia after germination begins. Experiments failed to show any sufficient evidence for diastase in the resting pollen grain of Zamia, and starch makes its appearance in these pollen grains only on germination. Diastase was absent from the pollen of Lupinus, Lathyrus, Eucharis, Richardia, and Narcissus. The diastase, according to Green, dissolves the starch without corroding the grains. The pollens tested for invertase were those of *Eucharis grandiflora*, *Narcissus papyraceus albus*, *N. Pseudo-Narcissus*, Helleborus, Richardia, *Lilium pardalinum*, and *Zamia Skinneri*. It was found in these, but was absent from the pollen of Alnus and of Clivia. He reports that

> A few experiments were made with a view to determining the existence of a cytolyst and a proteolyst, but in no case could either be found.

In the case of *Eucharis grandiflora*, tested for invertase, Green says that

> Only the contents of three or four anthers were used, yet a workable quantity of invertase was extracted.

In summarizing he says:

> The enzymes present in the resting pollen grains are, therefore, chiefly diastase and invertase, but their distribution is irregular, some containing one, some the other, and some both. At the onset of germination usually the amount of both diastase and invertase is considerably increased. . . . When the grain has lost the power of germinating the quantity of diastase is materially decreased.

The conclusions, as will be noted later, are not entirely in accordance with the results of the present experiments.

The Significance of Pollen to the Living Plant, and the Probable Rôle of the Pollen Enzymes

A medium-sized Indian-corn plant produces about 50,000,000 pollen grains. Cat tails (Typha), which produce about 60,000 flowers to the average spike, shed enormous quantities of pollen. A near relative, the elephant grass (*Typha elephantina*) of East India and New Zealand, yields enough for the natives to use as a flour in bread- and cake-making. The dense cloud of pollen from a pine tree has been photographed, and many a camper has noticed the yellow powder staining the canvas of his tent when dampness has moistened the grains. Liefmann (1904, p. 163) found 2,500,000 grains of grass pollen in one square meter. Yet so tiny and light are these pollen grains that a small amount represents millions of grains. Ulrich (1914) estimated 172,800,000 grains in one gram of ragweed pollen, and Kammann (1912) estimated 20,000,000 in one gram of timothy pollen. Pollen grains are nearly omnipresent during the flowering season. One would suppose from these figures that it is an easy matter to collect large quantities of pollen, but it is really not easy. The winged grains of pine pollen are blown away by the slightest breeze. Ragweed pollen cannot be collected easily after nine o'clock in the morning. The grain of pollen is surrounded by an oily envelope containing air. When this air is heated by the sun it causes the floating away of the pollen, or the so-called "smoking" of the ragweed. It is not easy to get enough for an experiment. The fact that during three fourths of the year we have pollen grains always with us makes it evident that if they have active enzyme action their importance cannot be lightly overlooked.

Pollen grains present many types of configuration. The commonest forms are oval or spherical, but an extreme variation is seen in the extraordinary filamentous pollen grains of eel grass (Zostera) and of another water plant, Halophila. Although the grains differ greatly in shape and in surface markings or finish, in internal structure they are very uniform. They usually consist in the Angiosperms of two cells. One cell is purely vegetative and gives rise to the pollen tube; the other is the generative cell.

Pollen grains vary considerably in size. A very extensive list of both measurements and descriptions of the pollen grains of many species and families is given by Hansgirg (1897, pp. 17–76).

The pollen grains are very resistant to excessive heat, cold, or dryness, and certain kinds retain their viability for many years. The pollen of the date palm tested by Popenoe at the Mecca experiment station was kept seven years and still retained its power of germination. Goodale (1916) found that dry pollen could retain its active poisonous properties for twenty-five to thirty years. It is evident that pollen is an interesting physiological unit, and our knowledge of its composition should be more complete.

Since one cell of the pollen grain is vegetative and gives rise to the pollen tube, food must be stored in the grain and at the time of germination rendered available. We should expect therefore to find enzymes suitable for the digestion of the materials stored in the grain, and perhaps capable of also digesting the inner pectin membrane (Mangin, 1893, p. 655) which envelops the grain. It is one aim of the experiments reported to determine whether such a correlation exists.

The distance that the pollen tubes have to traverse varies greatly. Where a style is absent and the stigmatic surface is just above the ovary, as in Vitis and Actaea, the tube has only a little way to penetrate. In flowers with long tubular corollas and slender filamentous styles, such as Crocus, Oenothera, and *Zea Mays*, the tubes attain a relatively great length. The time required for them to reach the ovule also varies greatly. In some flowers the tube reaches its full development in a few hours, while in the pine, following pollination in the spring, the grains put forth short tubes which do not complete their growth for a year (Kerner, 1895, **2**: 420). In certain oaks thirteen months elapse between pollination and fertilization. In regard to the Taxaceae, Coulter (1910, p. 268) writes:

> The tube may advance directly toward the archegonia or it may pursue a devious route, in some cases not reaching the archegonia until during the second season.

Other instances are cited by Coulter and Chamberlain (1903, p. 147). Why this long delay? An interesting physiological and chemical problem is waiting to be solved. The 13-inch pollen tube of *Colchicum autumnale* needs only twelve hours to reach its goal, and the 9-inch tube of *Cereus grandiflorus* completes its growth in a few hours (Schleiden, 1849, p. 407). In *Iris versicolor* the male nuclei were observed in the embryo sac 79 hours after fertilization and the tubes were 14 mm. long (Sawyer, 1917, p. 163). Surely an intruding, growing tissue of such size and duration must during its period of development, profoundly affect the cells with which it comes in contact, or which are adjacent to it, in its passage through the style. It has long been customary to liken the pollen tubes to the haustoria of parasitic fungi, for they closely resemble the latter in many respects. In Pinus, according to Mottier (1904), the tube serves both as a conducting passage for the male gamete and as an absorber of nutriment. The haustorial habit seems to be the more primitive condition, and we have survivals of it in certain Angiosperms, as in *Iris versicolor* (Sawyer, 1917), hazel, oak, elm, hickory, and certain mallows (Kerner, 1895), where the tube branches frequently and serves apparently as both haustorium and directing channel. (See also Coulter and Chamberlain, 1903, p. 148.) The nature of the tube has been dwelt upon here at such length in order to emphasize the fact that we ought to know more fully how these tubular filaments make their way through the tissues of the style and ovary. We assume that they digest their way. One author of a recent textbook even states positively:

> Very soon after pollination, the tube cell begins to develop a pollen tube, *which secretes an enzyme that dissolves the cell walls* and contents of the nucellar tissue, thus facilitating the passage of the delicate tube.

Is this true? Can we prove the existence of a cytase which digests the cell wall? Is one enzyme sufficient to account for the varied needs of the pollen tube in the course of its life history?

There are several conditions which the pollen tubes may encounter before they reach the embryo sac. These are as follows:

(1) *An open stylar canal.* In such cases the germinating tubes may force apart the cells of the stigma and soon enter the open space of the style without having to penetrate any cells, at least not until they reach the ovule. The middle lamella is usually composed of pectin compounds (Frémy, Mangin, Allen, and others). A pectin-digesting enzyme might therefore be required to dissolve the middle lamellae of the stigmatic cells, but afterwards the tube has a clear course. Examples of this sort are seen in violet, mignonette, lily, rhododendron, Hypericum, Cistus, *Atropa belladonna*, and iris. According to Kirkwood (1906), in the Cucurbitaceae

> The tubes pass chiefly over the surface of the conducting tissue lining the stylar canal and covering the placenta lobes, and this is rich in starch.

The suggestion is made that the tube is directed in its course by nutrient substances secreted by the conducting tissue. This would imply the presence of a diastase to digest the starch. Even if there is actually no tissue to be digested, it seems reasonable to suppose that the tubes may derive nourishment from the cells lining the stylar canal. Negative aërotropism, positive hydrotropism, and positive chemotropism, which have been frequently demonstrated in pollen tubes, direct their course so that they penetrate the stigma. These same responses tend in many cases to keep the tubes closely appressed to the cells lining the canal. Considering the length of time it often takes a tube to reach the ovule and its considerable growth, enzymes along with other factors in nutrition must play an important part. Frequently, as in Anagallis, the channel is only a narrow space almost completely filled with a mucilaginous substance, supposed to be secreted by the cells lining the canal. It may be pointed out here that the mucilages are closely related to the pectins. If this material is utilized by the tubes during their passage through it, we should expect a suitable enzyme to be present.

(2) *A mass of loose, conducting tissue in the style.* The cells in the interior

of the style frequently are loosely connected, elongated, and sometimes mucilaginous. The pollen tubes, according to most histological reports, penetrate the middle lamellae of these cells. This is the condition most frequently met with. The pollen tubes follow the middle lamellae of the cells throughout their course. The lamellae are, as has already been stated, composed either of pectin or of closely related mucilaginous substances. Here again the necessity for a pectin-digesting enzyme is evident. It has been sought for in the experiments reported later. Since this condition is the most common, many examples could be cited. It may be well seen in members of the grass family and in Salvia (Bower, 1919, p. 269). Histological evidence seems to indicate that the cells of the style often remain intact. Shreve (1906, p. 115) says in regard to the pitcher plant (*Sarracenia purpurea*):

> The pollen tubes grow between the cells of the stigmatic surface and their entire passage is between the cells of the conducting tissue and never through them.

Gow (1907, p. 136), describing the fertilization of skunk cabbage (*Spathyema foetida*), writes:

> The central portion of the style consists of a loose mass of thin-walled cells through which the pollen tube readily forces its way to the upper end of the ovary.

Miller's account of the growth of the pollen tube of corn through the silk or style is interesting (1919, p. 264):

> Each silk has two fibro-vascular bundles. These bundles are surrounded by sheath cells which are characterized by their dense contents and large flattened nuclei. It is *between* these cells that the pollen tube travels down the silk. Arriving at the base of the silk the pollen tube works its way *between* the sheath-like cells that extend from the fibro-vascular bundles of the silk to the cavity of the ovary. The tube enters the ovary and twists and coils in its passage along the ovule coat until it reaches the micropyle. The pollen tube then pushes *between* the cells of the ovule until it reaches the embryo sac.

Again, in another part of his account, he says:

> The end of the pollen tube is greatly enlarged as it pushes its way between the sheath cells of the bundle. In its passage down the silk the *tube causes but little disturbance in the position of the cells, so that after the tube disappears the cells quickly return to their normal form and position.* [The emphasis here is my own.] The pollen tube so far as I have observed does not extend the full length of the silk at any time. It is difficult to locate it a short distance back of its growing region. It appears that the older portions of the tube are absorbed by the surrounding cells, while the growing part of the tube is apparently nourished by the dense sheath cells.

Land (1907, p. 276), in explaining the fertilization of *Ephedra trifurca*, notes that the pollen tubes force their way between the neck cells of the archegonium, rarely destroying them in their passage. Only in two instances were the lower neck cells destroyed.

(3) *Cell walls penetrated by pollen tubes.* According to most investigators this condition occurs only rarely. Perhaps it will be found more frequent if more observations are made. The classic illustration is corn cockle,

Agrostemma. Strasburger's illustration of the tubes actually penetrating and half filling the papillar cells of the stigma has been frequently copied. Mallow pollen tubes do the same. Recently Knight (1918, entry 964) has reported that in the apple there is no stylar canal. "Pollen tubes make their way through the tissue. There is a decomposition of the cells along this path with the extrusion of mucilage." This is interesting to compare with the opinion of Grieg Smith that mucilages are decomposition products of cellulose, and with Wiesner's statement that all gums are produced by a diastatic ferment acting on cellulose. The writer regrets that it has been impossible to secure corn cockle and mallow pollen so as to determine whether their enzyme action is different from that of other pollens. Apple pollen has shown some differences. In histological studies of fertilization little attention seems to have been paid to the question of how much the pollen tube disorganizes the neighboring cells. It seems that it would be worth while to examine material again with this thought in mind. Many of the drawings of the passage of the pollen tubes appear very diagrammatic. In this connection it is interesting to note Kerner's observation (1895, p. 392) that the pollen tubes of *Lamium amplexicaule*

> Perforate the walls of the anther and grow in the direction of the stigma until they reach it.

Pollen Grains as Carriers of Bacteria and Molds

Nine varieties of pollen were tested to see if any contained a rennin-like enzyme, such as is found in the juices of a number of plants. Thymol had been added to the unheated and autoclaved pollen extracts, but the milk had not been sterilized. It was observed that both the unheated ragweed pollen and the autoclaved dock pollen control had strongly coagulated the milk over night at room temperature. Repetition of the test with highest grade milk (Fairlea Farm) showed that unheated corn, Easter lily, and dock pollens caused clotting, as did even the autoclaved dock pollen. The strong "youghourt" or fermented milk odor, and the behavior of dock pollen made the reaction seem more like bacterial than like enzymatic action. Apparently the single period of heating in the autoclave had not destroyed all bacteria on dock pollen. Accordingly a number of tests were made employing the usual bacteriological methods. These tests showed that pollen grains harbor a varied flora of both bacteria and molds. It had been taken for granted that excess of toluol or of thymol was sufficient to inhibit bacteria and molds. Do the results of these tests with milk mean that in other instances it is the enzymes of bacteria and molds rather than those of pollen grains which cause the change? The writer believes that this is not true for the following reasons:

a. The results were constant with the same pollen regardless of its source. Corn, pine, maple, and goldenrod pollen were collected both in New Haven, and, owing to the difference in seasons, a few weeks later on the hills of Vermont, six miles from a town. When this possible source of error was suspected, ragweed pollen was purposely obtained from Michigan, from two parts of New York state, and from Connecticut. It does not seem probable that the bacteria and molds carried by pollen can be so constant as to cause similar enzyme action in each instance.

b. The reactions are too rapid to be due to bacteria. With the inhibiting action of antiseptics the time required for bacteria to develop in sufficient numbers to produce similar changes would be much longer. All the enzyme reactions recorded have occurred within 24 hours, and several have been almost instantaneous.

c. Slices of wood in water over night are not in any degree sterile, yet bacteria which have free access do not destroy the middle lamellae, but pollen grains do. Pollen grains taken from unopened anthers and put into sterile Petri dishes are not likely to have peculiar bacteria, absent from the immediate environment. Besides, examination of the pollen contamination showed only a few omnipresent common forms of bacteria.

d. Pollen solutions filtered through a Berkefeld filter gave the enzyme action of diastase on starch, and blood fibrin digestion.

e. It is probable that the ground pollen added something to the milk which stimulated the growth of bacteria already in the milk, and that it was these which caused coagulation rather than the bacteria introduced by the pollen. The reason for this belief is that in all the plates poured from milk to which pollen had been added *Bacillus fluorescens liquefaciens* was the dominant type. The plates after standing a few days were a bright apple-green from the fluorescent growth.

On other plates poured later from the pollen extracts only, not once did this form appear. In the latter it was often not until the third or fourth day that colonies of molds occurred. Doubtless there are resistant forms of spores on the pollen which endure the heat of the autoclave and develop under favorable conditions on the agar plates, but these can hardly account for digestions which occur during twenty-four hours.

The Chemistry of Pollen

While many kinds of pollen have been examined for certain special constituents such as starch, nitrogen, phosphoric acid, etc., only eight kinds of pollen, as far as I have been able to ascertain, have been analyzed with any degree of completeness. Czapek (1905) discusses topically the occurrence and distribution of the principal constituents of plants; if a substance has been reported present in pollen he mentions the fact. These scattered references afford a valuable index to the original literature of the earlier analyses.

According to Heyl (1919*a*, p. 672) the walls of the pollen grain constitute 65 percent of the structure. Biourge (1892, p. 75) distinguishes four substances in the wall or envelope of pollen grains: cutin, cellulose, pectic substances, and callose. Sometimes one, or more than one, of the four materials are present in the same grain. These substances are indicated by characteristic solubility tests and by color reactions. He examined the pollen of 19 species of monocotyledons and 26 species of dicotyledons. His plates give over a hundred illustrations of the pollen grain coats and their sculpturing, showing details brought out by staining methods and chemical treatment. Mangin (1888, p. 144) states that the membrane is formed of pectin.

Water makes up a large but variable part of the grain. Thus Koessler (1918, p. 420) found 10.5 percent of moisture in ragweed pollen, while Heyl (1917, p. 1470) reports 5.2 percent for the same kind of pollen. Braconnot (1829, p. 104) found 47 percent of water in cat-tail pollen. Lidforss (1899, p. 292) examined a number of species and found the average moisture content to be about 10 percent.

The colors of pollen differ greatly. It is deep yellow in Easter lily, dark red in tiger lily, salmon in cypress, and white in petunia. Even in the same flower the color may vary, as is noted by Plimmer (1912, p. 51) in *Lythrum salicaria*, which has yellow pollen in the short stamens and bluish green pollen in the long stamens. Heyl (1919 *b*, p. 1285) states that the yellow pigment of ragweed is entirely glucosidic and about 0.6 percent of the pollen. He finds a quercitin glucoside which on melting yields a cherry-red oil; and a glucoside isorhamnetin which has beautiful characteristic crystals in the form of hexagonal prisms. So far, no other analysis of the pigments of pollen has been located in the literature.

Starch has been found present in some kinds of pollen and absent in others. Molisch tested 110 varieties and found starch abundant in 45, only a trace in 9 varieties, and absent from 46. That is, about half the kinds tested contained starch. Lidforss (1899, pp. 294–298) examined 150 wind-pollinated flowers of 72 genera and 29 families of native or naturalized Scandinavian plants, and found the pollen of all rich in starch. On the other hand, he tested the pollens of a few wind-pollinated tropical plants and found them starch-free. He also calls attention to the fact that Nägeli found the pollens of *Alnus glutinosa* and *Plantago lanceolata*, collected in Germany, starch-free, while pollens of the same species collected by himself in a more northerly region contained starch. Similarly, Nägeli found the pollen of juniper on Swedish mountains to be rich in starch, while Molisch found little in that of the Austrian juniper. Further, Molisch states that the pollen of *Antirrhinum tortuosum* is completely starch-free in summer, but in November he finds grains of three sorts, those which are normal but starch-free, little empty grains, and normal starch-containing grains. Tischler (1909, pp. 219–242), however, does not find this correlation between climate, or temperature, and the starch content of pollen. He examined a large number of tropical plants at Buitenzorg and reports that the plants growing under relatively unfavorable conditions of assimilation, for example on mountains 3,000 m. high and in the desert, showed no higher percentage of pollen with starch than the plants growing under the favorable climatic conditions of the tropical rain forest. He does, however, observe that there is frequently a difference in starch content between mature and immature grains.

Lidforss (1899, p. 306) reports the analysis of sixteen varieties of pollen for nitrogen and P_2O_5. Of these, 11 were from anemophilous, and 5 from entomophilous flowers. He found the average nitrogen content of the wind-carried pollen to be 4.63 percent, while that of the insect-carried pollen was 7.49 percent. The P_2O_5 showed a similar difference; the average for the former pollen being 1.76 percent, and for the latter 3.03 percent. Whether or not this represents a real correlation must be established by further observations.

The relative amounts of protein, fat, sugar, ash, etc., can best be seen by comparison of tables 1–7. It is interesting to note, from Stift's analyses of the pollen of three varieties of *Beta vulgaris*, that the different constituents may vary considerably in the pollen of one species (Stift, 1896, p. 43; 1901, pp. 105–106).

TABLE 1. *Comparison of Pollen Analyses (figures indicate percentages)*

Kind of Pollen	Authority	Protein	Fat	Ash	Carbohydrates	
Date palm..	Vauquelin, 1802			$Ca_3(PO_4)_2$ $Mg_3(PO_4)_2$		
Cat tail.....	Braconnot, 1829		3.60		Starch	Sugar
Cypress....	Church, 1875	8.67	1.87	3.70	85.76	
Hazel......	Planta, 1885	30.06	4.20	3.81	5.26	14.7 Saccharose
Pine.......	Planta, 1885	16.56	10.63	3.30	7.06	11.24
Pine.......	Kressling, 1891	15.87	10.00	5.50	7.40	12.075 Pentosans
Beet.......	Stift, 1896,	16.90	3.52	9.18	0.89	12.26
	1901	16.68	5.47	7.13	0.89	7.27
Rye........	Kammann, 1912	40.00	3.00	3.40	25	
Ragweed ...	Heyl, 1917	24.40	10.80	5.39	Dextrin 2.10	Sugars 2.10 Pentosans 7.26
Ragweed ...	Koessler, 1918	8.25(?)	10.30	10.60		6.89

Stoklasa (1896, p. 631) analyzed the pollen as well as various other organs of apple, horse chestnut, and beet, and concludes:

> Das lecithinreichste Organ der ganzen Pflanze aber ist entschieden das Pollenkorn.

He found in apple pollen 5.86, in that of horse chestnut 5.16, and in that of beet 6.04 percent of lecithin. Heyl (1919 *a*, p. 672) discusses the chemical "building stones" from which the substance of pollen sperm nuclei may be built, if there is a parallelism with the chemical composition of animal sperms.

TABLE 2. *Analysis of Pine Pollen, Przybytek and Famintzin, 1885 (figures indicate percentages)*

Water	6.79
Ash	
Calcium oxid	35.23
Sodium oxid	3.62
Magnesia	7.00
Calcium	0.88
Iron and aluminum oxid	5.30
Phosphoric acid (anhydrous)	29.86
Sulphuric acid (anhydrous)	14.83
Chlorine	0.99
Manganese	a trace

TABLE 3. *Stift's Analyses of Pollen from a Cattle-fodder Beet and from two Varieties of Sugar Beet (figures indicate percentages)*

	Fodder Beet, 1895	Sugar Beet, 1895	Sugar Beet, 1900
Protein	15.25	16.90	16.68
Nitrogenous substances not protein	2.50	2.77	5.82
Fat (ether extract)	3.18	3.52	5.47
Starch and dextrin	0.80	0.89	0.89
Pentosan	11.06	12.26	7.27
Other nitrogen-free extractives	23.70	26.27	28.86
Crude fiber	25.45	28.21	27.95
Ash	8.28	9.18	7.13
Water	9.78		

TABLE 4. *Heyl's Analysis of Ragweed Pollen (1917)*

Alcohol-soluble (42.9 percent) contains (in percentages):

Moisture	5.28
Starch (diastase)	0.00
Crude fiber	12.20
Pentosans	7.26
Protein	24.40
Nitrogen in alcoholic extract	1.08
Ash	5.39
Dextrin	2.10
Fat	10.80
Lecithin	0.75
Ether-soluble, but not ligroin-soluble	1.75
Sucrose	0.40
Glucose	1.60
Resin	17.40
A nitrogenous base	trace

From the above review and from the analyses given in tables 1–7 it is clear that our knowledge of the chemistry of the pollen of the very numerous species of flowering plants is very limited. It is a discouraging problem because of the difficulty of getting large quantities of material, as Heyl points out when he estimates that it takes 610 million grains of ragweed pollen to make a gram.

TABLE 5. *Kammann's (1912) Analysis of Rye Pollen (figures indicate percentages)*

Inorganic substances		13.58
Water	10.18	
Ash	3.4	
Organic substances		86.42
Alcohol-ether-soluble	3.	
Carbohydrate	25.	
Non-protein nitrogen	18.	
Protein	40.	

TABLE 6. *Koessler's (1918) Analysis of Ragweed Pollen (figures indicate percentages)*

Inorganic substances		21.1
Moisture	10.5	
Ash	10.6	
Organic substances		78.9
Total reducing sugars after hydrolysis	6.89	
Ether-soluble lipoids	10.3	
Fatty acids after hydrolysis	4.75	
Phytosterol	0.34	
Insoluble in ether but soluble in 95 percent alcohol	12.5	
Extractives, etc., soluble in alcohol (resins) and water	11.5	
Insoluble residue (crude fiber, proteins, etc.)	37.71	

TABLE 7. *Purin Bases and Amino Acids in Pollen*

Kind of Pollen	Authority	Purin Bases	Percentage
Pine	Planta, 1885	Hypoxanthine Guanine	0.04
Hazel	Planta, 1885	Hypoxanthine Guanine	0.15
Pine	Kressling, 1891	Xanthine Guanine Hypoxanthine	0.015 0.021 0.085
		Amino Acids	
Ragweed	Heyl, 1917	Histidine Arginine Lysine Agmantine	not given " " "
Ragweed	Koessler, 1918	Arginine Histidine Cystine Lysine	2.13 2.41 0.57 0.97

Other Physiological Aspects of Pollen in which Enzymes may Play a Part

In certain flowers there are two kinds of pollen grains, some of which produce tubes and others which do not. Müller (1883, p. 242) first distinguished these as "*Befruchtungs*"- and "*Beköstigungs*"-pollens, the former being the fertile, and the latter the sterile pollen which Müller thought served as the food of the pollinating insects. Tischler (1910, pp. 219–242) has studied this subject and has made the interesting discovery that in certain pollens, at least, the sterile grains may be stimulated to produce tubes by the addition to the culture medium of a trace of saliva or of diastase. The lack of a specific enzyme in these pollens seems thus to be the cause of sterility. It is quite possible that in other pollens the lack of pectinase, cytase, invertase, or of other enzymes may be equally important in inhibiting the growth of the tube. In some cases the deficiency may be made good by an enzyme secreted by the stigma. The whole question has a great deal of significance in problems of plant breeding.

Pollen enzymes may be concerned in the production of the characteristic odors of pollen which are probably factors in insect attraction. The emanations from moist pollen indicate the presence of fermentation products.

It also seems reasonable to suppose, as Erlenmeyer (1874, p. 206) has suggested, that pollen enzymes are co-workers with the enzymes from the body of the bee used in producing bee-bread.

Gardeners commonly believe that contact with pollen is frequently the cause of the discoloration and decomposition of the petals which is often a sequence of pollination.

II. Experiments in Regard to Pollen Enzymes

Plan of the Experiments

An effort has been made to collect a large variety of pollens, representing different families of plants, and including some of the so-called "hay-fever pollens." These pollens have been tested for twelve different enzymes. On account of the difficulties in collecting all the pollens at the start, the experiments have been made in two series. For the first the available pollens were those of (1) Easter lily, (2) *Lilium rubrum*, (3) red maple, (4) Norway maple, (5) Siberian crab-apple, (6) Austrian pine, (7) Scotch pine, (8) magnolia, and (9) dandelion. In the second series of experiments, in addition to some of the first nine pollens, those of the following plants were used: (10) corn, (11) daisy, (12) dock, (13) elm, (14) goldenrod, (15) ragweed, (16) rye, (17) tiger lily, (18) timothy. Not every one of the eighteen pollens has been used in every test, but an effort has been made to use as many as possible.

Methods of Collecting Pollen. Kinds of Pollen Used

The work was begun in February. At this time Easter lily pollen was available in the largest quantity. Since it is customary to remove the anthers as the flower opens, to prevent the pollen from staining the petals, it was easy to find an obliging florist who would place these anthers in a clean paper box. In this way surprisingly large quantities of pollen were secured. Care had to be taken to prevent molding. A paper box was found to be better for collection than glass jars, as the anthers dried more readily. It was also necessary to keep the anthers spread out, and to place them in a sulphuric-acid desiccator as soon as possible after collection.

When the anthers are dry, or partially dry, the large, sticky yellow pollen grains easily fall out. They can then be accumulated quickly by placing the anthers on one half of the bottom of a petri dish, moistening the other half with the finger tip, and then when the dish is covered and shaken in a horizontal plane the pollen adheres and heaps up on the moistened surface.

When it was necessary to remove adhering masses of pollen from a dish a glass brush was found better than a camel's hair brush, and for this purpose the glass brush from a Beegee ink eraser was excellent.

The easiest way of collecting the tiny pollen from many small flowers is by drying the blossoms on large sheets of paper and shaking them through a fine sieve. The anthers usually sift out and the pollen can be separated from the anthers by sifting again through fine silk bolting cloth. (Mimeograph typewriter diaphragm silk is convenient.) The microscope showed, in the case of red maple, that invisible hairs from the flower also sifted through, but the pollen from other plants appeared quite free from foreign particles.

Wodehouse (1916, p. 430) has suggested an excellent way of collecting large quantities of ragweed pollen.

> The flower heads just coming into bloom are crushed in a mortar with several volumes of carbon tetrachlorid. When strained through muslin the pollen passes through with the CCl_4 and can be separated by filtering on filter paper. The pollen is lighter yellow since the CCl_4 probably removed lecithin.

In collecting pine pollen it was found necessary to gather the staminate cones before they had opened, because later the slightest shaking of the branch scattered a cloud of pollen to the four winds. Cutting off the tassels of corn and allowing them to open indoors, over large sheets of paper, undisturbed by currents of air, gave the largest yield of corn pollen.

Preliminary Experiments

These experiments were in two parts: (1) Germination of the pollen grains, and (2) Comparison of the enzyme action of unground, ground, and germinated pollen. The results of these tests showed that the pollen ground with powdered glass was more effective in its enzyme action than either the unground or even the germinated pollen. The experiments were made as follows:

To secure vigorous growth of pollen tubes, Easter lily pollen was germinated (1) in tap water, (2) in 3, 5, and 16 percent sugar solution, (3) on agar, and (4) in Knop's solution and modifications. The stock agar recommended by Crabill and Reed (1915, p. 2) was used. This contains no carbon-containing nutrient and therefore does not favor bacterial and mold growths, which are exceedingly troublesome.

Formula for Stock Agar

Distilled water	1,000 cc.
Magnesium sulphate	0.5 g.
Di-potassium hydrogen phosphate	1.0 g.
Potassium chlorid	0.5 g.
Ferrous sulphate	0.1 g.
Agar	2.0 g.

The pollen tubes grew exceedingly well in the film of moisture formed on the surface of agar in petri dishes. The tubes were thicker, appeared more vigorous, and showed protoplasmic movement better than when grown in water or in dilute sugar solutions. This might be used as a method of showing variation in cell turgescence according to the density of the medium.

Formula for Knop's Solution

K_2SO_4	0.7 g. in 1 liter of water
NaCl	0.23 g.
$CaSO_4$	0.7 g.
$MgSO_4$	0.5 g.
Na_3PO_4	0.5 g.
NH_4NO_3	(solution 0.0649) 20 cc.

The tubes grew best in solutions from which the K_2SO_4 was omitted, and best of all in one in which the $CaSO_4$ was increased to 1.0 g. The K_2SO_4 seemed to cause disintegration of the tubes after 48 hours, but this evidence is of course very slight and more experiments must be tried to prove anything.

Of the four media used, tap water was selected as the best for Easter lily pollen. The grains germinated and produced long tubes in 24 hours, and the solution contained no foreign matter to be taken into consideration. After the tubes were well grown the pollen mass was filtered and dried in a desiccator. This dried germinated pollen was used both unground and ground with powdered glass.

Comparative quantitative determinations of the enzyme action of the unground, ground, and germinated pollen were made as follows: Having previously noted the marked invertase action of Easter lily pollen on cane sugar, the amount of copper precipitated from Fehling's solution by the reducing sugar formed was taken as an index of enzyme action.

The tests were made in five test tubes as follows: In each tube were placed 300 mg. of cane sugar, 15 cc. of distilled water (except in tube 4, where 10 cc. was used), and 8 drops of toluol. To tubes 1, 2, and 3 were added respectively 300 mg. each of unground, ground, and ground germinated pollen. To tube 4 was added 300 mg. of pollen boiled in 5 cc. of water, making the total quantity the same as in the other tubes. Tube 5 had no pollen added and served as a second control.

These tubes were allowed to stand in a warm room for 24 hours and were shaken occasionally. After this interval, 15 drops of each of the five suspensions was taken. To each portion 15 cc. of fresh Fehling's solution was added. The tubes were placed in a water bath and boiled an hour. The solutions were then filtered on desiccator-dried, weighed filter paper, and the copper precipitate was washed with hot water until free from the excess of Fehling's solution. The filter papers were then dried first in an oven and then in a desiccator and again weighed. The gain in weight represents the amount of reducing sugar present.

The weights of the papers are shown in table 8.

Table 8

	1st Weight	2d Weight	Gain
Unground pollen	824 mg.	857.05 mg.	33.05 mg.
Ground pollen	832 mg.	860.2 mg.	28.2 mg.
Ground germinated pollen	831.5 mg.	857 mg.	25.5 mg.
Boiled pollen	843 mg.	845.35 mg.	2.35 mg.
Sugar solution only	828 mg.	829.1 mg.	1.1 mg.

The gain in the unground pollen, which appears larger, is relatively less because in this test the 300 mg. was all pollen, while the 300 mg. in the other tests was partly powdered glass. From these figures and from several similar tests it seemed evident that in the case of Easter lily pollen invertase, at least, there was no advantage in previously germinating the pollen grains. Repetition of this type of experiment might show a wide range of variation both for kinds of pollen and for their enzymes.

The data obtained in testing for pectinase in Easter lily pollen confirmed the opinion that for this kind of pollen there was no gain in pectinase as a result of germination.

Tests for Amylase

The method used was to test a known quantity of starch paste with active pollen and an equal quantity with boiled pollen for a control. First, 10 cc. of 1 percent starch paste was used with 150 mg. of pollen. Later, 5 drops of 1 percent starch in 10 cc. of water was found to be a better dilution. Toluol was used as an antiseptic. The tubes were allowed to stand in a warm room for 24 hours and were shaken occasionally. Two portions of 15 drops each were then taken from each tube, and to one was added 2 drops of iodine to see if the starch had disappeared, and the other was heated with 15 drops of Fehling's solution to see if sugar had appeared. The results are seen in table 9.

In these tests, as in those already mentioned, the ground pollen was more active than the unground, but the germinated pollen did not appear to be more active than the ungerminated.

From table 10 it is seen that all kinds of pollen tested contained an amylase, but that this amylase was less active in the apple pollen (Siberian crab) and in that of the magnolia (cucumber tree) than in the other kinds.

In later tests with other kinds of pollen, Benedict's solution was used instead of Fehling's solution, as it is a more delicate test.

TABLE 9. *Tests for Amylase in Easter Lily Pollen*

	10 Cc. of 1 Percent Starch Solution + Toluol	5 Drops of 1 Percent Starch Solution in 10 Cc. H_2O + Toluol
Fresh (unground)	Slight digestion	Complete digestion
Fresh (unground)	Marked digestion but not complete	Complete digestion
Germinated	Marked digestion but not complete	Complete digestion
Germinated (ground)	Nearly complete digestion	Complete digestion
Boiled pollen	No digestion	No digestion

TABLE 10. *Tests for Amylase in Different Kinds of Pollen*

Pollen, 150 mg., added to 5 drops of 1 percent starch solution in 10 cc. of water to which toluol was added as an antiseptic.

Tests for starch: 15 drops of starch solution + pollen + 2 drops of iodine.

Kinds of Pollen	Active Pollen	Boiled Pollen
Easter lily	Rapid digestion	No digestion
Lilium rubrum	" "	" "
Red maple	" "	" "
Norway maple	" "	" "
Apple, Siberian crab	Slight digestion	" "
Austrian pine	Rapid digestion	" "
Scotch pine	" "	" "
Cucumber tree	" "	" "
Dandelion	Slow digestion	" "

Tests for sugar: 15 drops of starch solution + pollen, heated with 15 drops of Fehling's solution.

Kinds of Pollen	Active Pollen	Boiled Pollen
Easter lily	Rapid reduction	Some reduction
Lilium rubrum	" "	" "
Red maple	" "	" "
Norway maple	" "	" "
Apple, Siberian crab	Some reduction after ½ hr. heating	No reduction Cf. Table 8
Austrian pine	Rapid reduction	Some reduction
Scotch pine	" "	" "
Cucumber tree	" "	" "
Dandelion	" "	" "

Tests for Reducing Sugars

Since all the controls in the tests of amylase, except the boiled apple pollen, gave some reduction of Fehling's or of Benedict's solution, tests were made to determine the kind of sugar present in pollen. Filtered water extracts of the kinds of pollen listed above were heated with Fehling's solution. All except the apple pollen were found to contain reducing sugars, or some easily oxidized substance. When the apple-pollen extract was hydrolyzed with HCl and then neutralized with NaOH, it reduced the Fehling's solution, indicating the presence of a sucrose.

Tests for Starch

Solutions were treated first with chloral hydrate to render the grains transparent, and afterwards with iodine.

TABLE 11. *Tests for Starch in Different Kinds of Pollen*

1. Apple	–	10. Pine, Austrian	–
2. Corn	+	11. Pine, white	–
3. Daisy	–	12. Ragweed	–
4. Dandelion	–	13. Rye	+
5. Dock	+	14. Timothy	+
6. Elm	+	15. Magnolia	–
7. Goldenrod	–	16. Maple, Norway	–
8. Lily, Easter	–	17. *Lilium rubrum*	–
9. Lily, tiger	–	18. Maple, red	–

Tests for Zymase

The different kinds of pollen were tested with Pasteur's fluid, in Smith's fermentation tubes, for zymase. Toluol was added to inhibit bacterial action or molds. Apple pollen was the only one which showed any reaction, and since this was after standing 48 hours the result was doubtful. Since, however, apple pollen has been an exception in other instances, this test will be repeated when more pollen is available.

Tests for Invertase

Equal amounts of the different kinds of ground pollen (about 150 mg.) were added to 5 cc. of 3 percent cane sugar solution with 5 cc. of distilled water and 8 drops of toluol. Equal amounts of ground pollen were boiled with 5 cc. of distilled water and added to 5 cc. of the cane sugar with 8 drops of toluol solution for controls. The two sets of tubes were allowed to stand for 24 hours in a warm room. Then to 15 drops of each pollen solution were added 15 drops of Fehling's solution and the tubes were heated ½ hour to 1 hour in a boiling water bath, and the rate and amount of reduction in the different tubes were observed. Although this was not an exact quantitative test, as for the Easter lily pollen, yet the varying amounts of reduction in the different pollen solutions, and the differences between the active solutions and the controls, were strikingly noticeable. When the pollen was acid, producing a green color in Fehling's solution before heating, the tests were repeated, neutralizing the solution first. This was marked in red maple. Since the active pollen in every case caused more reduction than the boiled control, the reduction could not have been due merely to the reducing sugars of the pollen grains since the ruptured boiled grains would have yielded just as much sugar. The difference, therefore, may be due to the action of invertase. Moreover, apple pollen, which was found to contain sucrose, was extremely active in its invertase reaction. The results are shown in table 12.

TABLE 12. *Tests for Invertase*

Kinds of Pollen	Active Pollen	Control
Easter lily pollen	Rapid reduction	Slight reduction
Lilium rubrum	Slow "	" "
Red maple	Very rapid reduction	" "
Norway maple	Rapid reduction	" "
*Apple	Instant "	Some after 20 min. heating
Austrian pine	Slow but marked	Slight reduction
Scotch pine	" " "	" "
Magnolia	Very rapid	" "
Dandelion	Slow but marked	" "

Tests for Lipase

In the different methods used for testing for lipolytic enzymes the following substrates and testing reagents were used:

1. *Substrates.*

(1) Ethyl butyrate.

(2) Olive oil acidified with decinormal acetic acid and a little gum arabic added to make an emulsion.

(3) Olive oil emulsion recommended by Zeller. 10 cc. of olive oil was dissolved in hot 100 percent alcohol. This was run through a hot separating funnel to which was attached a piece of glass tubing drawn out to a capillary jet. The stream of oil in alcohol was run into 100 cc. of cold distilled water which was stirred continually. The milky emulsion was then heated to drive off the alcohol and afterwards diluted with water.

(4) Methyl acetate.

2. *Activator.* Approximately N/60 oxalic acid was used, partly because free acid is needed to counteract the slight alkalinity of the ground glass and more especially because free acid accelerates the activity of lipase.

3. *Alkali for titration.* Approximately N/10 sodium hydroxid solution was used to which a trace of barium hydroxid was added. To insure uniformity in readings, a 3-liter bottle was filled, and the solution was drawn off as needed through a connected graduated burette. Both the bottle and the burette had soda-lime bulbs at the inlet to absorb CO_2.

4. *Indicator.* Phenolphthalein was used in all titrations as an indicator.

5. *Antiseptic.* Toluol was added as an antiseptic. Controls of autoclaved pollen extract were run in each case, and the digestions were carried on in small stoppered Erlenmeyer flasks kept in an electric incubator at 36°–38° C. Samples were titrated at different time intervals. Methyl acetate was more strongly hydrolyzed than either ethyl butyrate or the olive oil preparations.

Austrian pine, dock, daisy, goldenrod, ragweed, rye, and timothy pollens were tested with the different substrates for lipase. The tests with ethyl butyrate were unsatisfactory. In the olive oil emulsion and methyl acetate media, Austrian pine, dock, ragweed, and rye pollens gave positive tests for lipase. The action on methyl acetate was especially marked with Austrian pine pollen, in which case the titrations showed nearly double the amount of acid with fresh pollen as compared with the boiled control.

Tests for Proteolytic Enzymes

Substrates for Proteolytic Enzymes

1. *Blood fibrin.* Fresh fibrin from pig's blood was obtained at the slaughter house, and was washed for several hours with a stream of cold water to remove corpuscles. Fairly uniform and compact strands of the fibrin were selected, and portions as nearly equal as possible were placed in test tubes with 10 cc. of distilled water, plugged with cotton, and sterilized for 20 minutes in an autoclave. Other portions were stained with 1 percent Congo red and the color was fixed by immersion in boiling water. The red color is liberated when the fibrin is digested. The colored fibrin was also sterilized.

TABLE 13. *Fermi's Gelatin Test*

5 cc. Fermi's gelatin, 5 cc. H_2O, 100 mg. pollen, 37° C. Degrees of liquefaction or failure to solidify, after standing in ice water 10 minutes, indicated by signs.

Kind of Pollen	Unheated Pollen		Autoclaved Pollen	
	24 Hrs.	48 Hrs.	24 Hrs.	48 Hrs.
1. Apple	+	++	−	−
2. Corn	+	+	−	−
3. Daisy	+	+	−	−
4. Dandelion	+	+	−	−
5. Dock	++	+++	−	−
6. Elm	+	++	−	−
7. Goldenrod	+	++	−	−
8. Lily, Easter	++	+++	−	−
9. Lily, tiger	++	++	−	−
10. Pine, Austrian	+	+++	−	−
11. Pine, white	+	++	−	−
12. Ragweed	+++	++++	−	−
13. Rye	++	++	−	−
14. Timothy	+	+	−	−
15. Magnolia	++	+++	−	−
16. Maple, Norway	+	+	−	−

2. *Fermi's gelatin.* The proportions used were those given by Dernby. 700 grams of gelatin were dissolved in 1,250 cc. of hot water over a water bath, strained through cheese cloth, and 2 grams of finely pulverized thymol were added. The solution was diluted to 2 liters and sterilized. Dernby diluted further before using, but this was not found necessary with the pollen extracts. When the gelatin was used it was melted over a bath and

to 5-cc. portions were added 5 cc. of distilled water and 100 mg. of pollen. For the control the pollen and water were first autoclaved. The tubes were incubated at 37° C. for 24 hours or longer (see table 13). The tubes were taken from the incubator and placed simultaneously in ice water, and the failure to solidify, or degree of congealing, during 10 minutes was noted.

Many investigators have used gelatin for detecting enzymes of the pepsin type, but the experiments of Malfitano, Mavrofannis, and Jordan, recently confirmed by Berman and Rettger, seem to indicate that liquefaction of gelatin by an organism is not proof of proteolytic activity. However, since pollen extracts, like pineapple juice, possess this power of liquefaction to a marked degree it has been considered worth while to record the observations.

Tests for Trypsin

Table 14

Congo red, blood fibrin, 2 cc. N/10 Na_2CO_3, 10 cc. pollen extract (50 mg. in 100 cc. distilled water, unheated and autoclaved), 1 mg. thymol in each tube.

Kind of Pollen	Test 24 Hrs.	Appearance of Solutions	
		Unheated	Autoclaved
1. Apple	—	No change	No change
2. Corn	++	Fibers disintegrated. Liquid very pink	Fibers unaltered Liquid pale yellow
3. Daisy	—	No change	No change
4. Dandelion	—	" "	" "
5. Dock	?	Fibers less firm. Liquid pinkish	No change
6. Elm	?	Liquid faint pink	" "
7. Goldenrod	++	Fibers disappeared. Liquid red-brown	Fibers unaltered Liquid yellow-brown
8. Lily, Easter	—	No change	No change
9. Lily, tiger	—	" "	" "
10. Pine, Austrian	+	Fibers partly disintegrated, liquid pink	No change
11. Pine, white	+	Fibers partly disintegrated, liquid pink	No change
12. Ragweed	+	Fibers partly disintegrated, liquid pink	No change
13. Rye	+	Fibers partly disintegrated, liquid pink	No change
14. Timothy	+	Fibers partly disintegrated, liquid pink	No change
15. Magnolia	++	Fibers disintegrated, liquid pink	No change
16. Maple, Norway	—	No change	" "

From tables 14–16 it may be seen that corn, goldenrod, Austrian pine, white pine, ragweed, rye, and timothy all gave positive results. Dock gave strongly positive results in the less alkaline medium, while magnolia and goldenrod were strongly positive in the more alkaline medium. Apple was negative with Na_2CO_3 added, but was positive without the addition of

TABLE 15

Same as shown in table 14, except that no Na_2CO_3 was added. Solutions slightly alkaline from the powdered glass. Thymol added to each tube.

Kinds of Pollen	Test 24 Hrs.	Appearance of Solutions	
		Unheated	Autoclaved
1. Apple	+	Fibers slightly disintegrated. Liquid pink	No change
2. Corn	++	Fibers completely disintegrated. Liquid pink	" "
3. Daisy	?	Fibrin darker, liquid milky	" "
4. Dandelion	—	No change	" "
5. Dock	++	Fibers completely disintegrated. Liquid pink	" "
6. Elm	?	Slight turbidity	" "
7. Goldenrod	+	Fibrin shrunken. Liquid dark brown	" "
8. Lily, Easter	—	" " " " "	" "
9. Lily, tiger	—	" " " " "	" "
10. Pine, Austrian	+	Disintegration, turbid, pink	" "
11. Pine, white	+	" " "	" "
12. Ragweed	+	" " "	" "
13. Rye	+	" " "	" "
14. Timothy	++	Complete disintegration. Liquid red	" "
15. Magnolia	—	No change	" "
16. Maple, Norway	?	Liquid turbid pinkish	" "

Tests for Pepsin

TABLE 16

Same as shown in table 14, except that 2 cc. of 0.2 percent HCl was added instead of Na_2CO_3.

Kind of Pollen	Test 24 Hrs.	Appearance of Solutions	
		Unheated	Autoclaved
1. Apple	—	No change	No change
2. Corn	+	Disintegration. Liquid pink	" "
3. Daisy	—	No change	" "
4. Dandelion	—	" "	" "
5. Dock	—	" "	" "
6. Elm	—	" "	" "
7. Goldenrod	—	" "	" "
8. Lily, Easter	—	" "	" "
9. Lily, tiger	—	" "	" "
10. Pine, Austrian	—	" "	" "
11. Pine, white	—	" "	" "
12. Ragweed	—	" "	" "
13. Rye	+	Disintegration. Liquid pink	" "
14. Timothy	+	" " "	" "
15. Magnolia	—	No change	" "
16. Maple, Norway	—	" "	" "

alkali. It may also be noted that in the digestion of fibrin in the presence of 0.2 percent HCl only the Gramineae showed activity. When $Na_2 Co_3$ was left out, apple, daisy (?), and dock were added to the list. These tests were repeated several times and gave consistent results. Toluol was substituted for thymol without any noticeable difference. In no case was there the slightest odor of putrefaction. The antiseptics were easily detected by their odor.

Tests for Erepsin

Solutions of Witte's peptone were used in the following proportions:

1. 10 cc. of 1 percent Witte's peptone, 2 cc. of N/10 sodium carbonate, 5 cc. of pollen extract 50 mg. in 10 cc. (unheated and autoclaved), 100 mg. of thymol.
2. 10 cc. of 1/10 per cent Witte's peptone, 1 cc. of N/10 sodium carbonate, 10 cc. of pollen extract (unheated and autoclaved), 100 mg. of thymol.
3. The above described solutions were used without adding sodium carbonate.

For testing, Gies's biuret reagent was used. This reagent consists of 10 percent KOH solution, to which 25 cc. of 3 percent $CuSO_4$ solution per liter is added. A large flask was filled with the reagent and connected with a graduated burette so that for each test the same strength of reagent should be used. In making the tests, 1 cc. of the solution to be tested was put with 20 cc. of biuret reagent in 25 cc. Nessler comparator tubes of uniform diameter and thickness. The color differences were read by looking down through the liquid at a white background. Solution 2 proved the best dilution. More than 1 cc. of the pollen-peptone solution did not give satisfactory results because of color interference and turbidity. In each test three tubes were compared: (1) 1 cc. peptone solution, or peptone plus Na_2CO_3, and thymol. (2) 1 cc. peptone, or peptone plus Na_2CO_3 plus unheated pollen, and thymol. (3) 1 cc. peptone, or peptone plus Na_2CO_3 plus autoclaved pollen.

The sixteen varieties of pollen previously listed were tested, but only apple and magnolia pollen gave positive results. Here the reaction of the unheated pollen with the biuret reagent gave a very faint pinkish tint as compared with the rose-violet tint of the controls.

Tests for Catalase

The decomposition of hydrogen peroxid in a fermentation tube was used as an indication of a catalase. All the kinds of pollen tested showed a marked reaction. Easter lily, magnolia, and apple pollen were exceedingly active. Maple pollen was the slowest but the action was evident. The boiled pollen extracts did not give the reaction.

Tests for Reductase or a Reducing Substance

The reduction of potassium permanganate solution by the different kinds of pollen was tested. All showed some reducing action; apple, Austrian pine, and magnolia were especially active. Apple pollen changed $KMnO_4$

at once to a pale amber. The boiled controls did not reduce the permanganate. This is not necessarily indicative of enzyme action. The reduction may be brought about by substances produced by the decomposition of the pollen grains.

Tests for Nuclease

Tests for phosphoric acid, which might indicate the splitting of nucleic acid, were made with ammonium molybdate on ground, unground, germinated, and boiled Easter lily pollen. All showed a strong phosphoric acid test so that no conclusions could be drawn.

Tests for Tyrosinase

A solution of tyrosin gave a negative reaction for all pollens of the first series tested.

Tests for Laccase

An alcoholic solution of gum guiacum, which was rapidly colored blue by freshly cut potato or orange peel, gave negative results with ten varieties of pollen.

Tests for Cytase

1. The method given by Crabill and Reed was used. Filter paper is dipped in manganese sulphate solution and then in potassium permanganate solution. The resulting manganic oxid colors the paper dark brown. Acids formed by the cellulose destruction combine with the manganic oxid to form light-colored salts which show by contrast on the brown background. Both ground and unground pollen placed on moistened sterilized strips of such paper caused no color change, although a subsequent growth of mold did so.

2. Cellulose was prepared from filter paper in the following manner. Schweitzer's reagent (ammoniacal cupric oxid) was used as a solvent. This was made by adding to a strong copper sulphate solution, first, ammonium chloride, and then an excess of sodium hydroxid. The blue-green precipitate thus formed was allowed to settle, the liquid decanted off, and the precipitate washed repeatedly with water on a Buchner funnel and filtered by suction. The precipitate was then dissolved in 0.92 percent ammonia. The resulting deep blue liquid readily dissolves strips of filter paper. When sufficient paper had been dissolved to make a thick, syrupy liquid, it was poured into dilute hydrochloric acid (1 : 5) and the cellulose was precipitated in small flecks. The precipitated cellulose was washed repeatedly with water on a Buchner funnel and filtered by suction, until the filtrate showed no trace of HCl when tested with $AgNO_3$. The pure white mass of cellulose was then boiled with distilled water to make a fine suspension and to sterilize it. The suspension was tested with both iodine and Benedict's solution to be sure that it was both starch- and sugar-free, as was the case. The tests were made as follows: 10 cc. of cellulose suspension, 100 mg. of ground

pollen, 30 cc. of distilled water, and 2 cc. of toluol were placed in small stoppered flasks. For the control the pollen and water were heated in the autoclave. The suspensions were incubated at 37° C., and shaken frequently. The solutions were tested for sugar at 24-, 48-, and 96-hour intervals, and then allowed to stand for several weeks at room temperature to see if there would be complete destruction of the cellulose. In no case has this occurred, although several preparations have been kept for three months (see table 17). In testing for sugar the flasks were well shaken. Then the cellulose was allowed to settle out and 2 cc. of the clear liquid was transferred to a test tube and 10 cc. of Benedict's solution was added. All the tubes, both the unboiled and the boiled pollen controls, were then heated simultaneously in a boiling water bath until reduction was complete. If there was a striking difference in the amount of precipitate in the unboiled and in the control the quantitative test was made, but if no difference could be detected the precipitates were weighed as described on a previous page. Pollens were selected which did not all contain starch, so that the resulting gain in sugar did not come from this source. Yet the diminution in the amount of cellulose in the flasks was so slight that it is difficult to interpret results.

TABLE 17

Kinds of Pollen	Unheated Pollen	Autoclaved Pollen
1. Apple	+	—
2. Corn	—	—
3. Daisy	—	—
4. Dandelion	—	—
5. Dock	+	—
6. Elm	—	—
7. Goldenrod	—	—
8. Lily, Easter	—	—
9. Lily, tiger	—	—
10. Pine, Austrian	+	—
11. Pine, white	+	—
12. Ragweed	—	—
13. Rye	+	—
14. Timothy	+	—
15. Magnolia	—	—
16. Maple, Norway	—	—

Tests for Pectinase

As has already been noted, special importance was attached to the possible occurrence of a pectinase as indicative that the pollen tube digests the inner lamella of pectin in the cell walls of the pistil. Three methods of testing for a pectinase were tried.

1. Pistils of Easter lily were placed in a tube with distilled water and freshly ground pollen, and a second set were similarly treated with boiled pollen. After 24 hours the pistils were examined for alteration of texture.

It was found that the pistils with the active pollen had noticeably softened and the styles could easily be teased apart with dissecting needles, while the pistils with boiled pollen were still firm. The longitudinal and cross sections of the pistil treated similarly showed the same results. Incidentally, the chemotropism of the pollen grain for the stigma was conspicuous in the active pollen tubes.

2. Equal amounts of desiccator-dried ground Easter lily pistil were treated with pollen, and tested quantitatively for sugar as in the invertase test already described.

Dried Ground Pistil	Boiled Pollen	Water	Toluol
(*a*) 150 mg.	100 mg. in 5 cc. water	10 cc.	4 cc.
"	100 mg. ungerminated	15 cc.	"
"	100 mg. germinated	"	"
"	100 mg. ground	"	"

(*b*) Solution 15 drops, alkaline to neutral.
Fehling's solution 15 drops.
Boil in water bath ½ hour.
Filter on weighed, desiccator-dried paper.
Dry in oven and desiccator.
Weigh again.

	1st Weight (mg.)	2d Weight (mg.)	Gain (mg.)
(*c*) Boiled pollen	711	716.3	5.3
Ungerminated	711.2	720.15	8.95
Germinated	720.28	729.00	8.72
Ground	827.00	839.00	12.00

3. The action of pollen on pectin was tested. The pectin used for the tests was prepared from the white inner skin of grape fruit, as follows:

Remove the white and boil in water.
Put through a meat chopper.
Leave in cold water 24 hours.
Boil from ½ to 1 hour.
Strain through cheesecloth.
Filter.
Concentrate by heating over a water bath.
Add 95 percent alcohol to precipitate.
Filter.
Wash with absolute alcohol.
Dry in a desiccator.

For the tests the following amounts were used:

(*a*) 3 cc. of pectin solution (300 mg. in 20 cc. of water), 300 mg. of pollen, 15 cc. of water, 8 drops of toluol.

(*b*) 15 drops of the pollen-pectin solution, 15 drops of Fehling's solution, or Benedict's solution. Treat as stated above (2 *b*).

	1st Weight (mg.)	2d Weight (mg.)	Gain (mg.)
(*c*) Austrian pine (active)	767.5	789	21.5
" " (boiled)	779	785	6
Norway maple (active)	778	797	19
" " (boiled)	782	794	12
Magnolia (active)	814	835	21
" (boiled)	809	815	6
Apple (active)	795	805	10
" (boiled)	799	807	8

(*d*) In this test 150 mg. of pectin and 150 mg. of ground pollen and glass were used, and the entire amount precipitated with Fehling's solution.

	1st Weight (mg.)	2d Weight (mg.)	Gain (mg.)	Actual Gain (mg.)	
Apple pollen (active)	765	963	198(−150)	48	28
" " (boiled)	754	924	170(−150)	20	
Red maple (active)	767	969	202(−150)	72	23
" " (boiled)	748	947	199(−150)	49	

(*e*) Later similar tests were made with seven other kinds of pollen, daisy, dock, goldenrod, white pine, ragweed, rye, and timothy. All gave positive results for the pectinase test.

Subtracting the constant 150 mg. of pollen and glass, the actual gain of sugar from pectin for the apple pollen, as compared with the control, is 28 mg. and for the red maple pollen 23 mg. This is larger than in the previous table, but larger quantities were used. Not only do the quantitative determinations show that pectin is converted into sugar by active pollen, but also in comparing the tests and their controls in the test tube it was very noticeable that the reduction of Fehling's solution was greater with unboiled pollen.

4. Another test which confirmed the presence of a pectinase was made for me by Mr. F. B. H. Brown, Yale University. Mr. Brown in his work on tropical woods, by a special method of technique, has succeeded in making sections one eighth as thick as are usually cut. When sections of dragon-tree wood (*Dracaena aurea*), *Tecoma obtusà*, and a species of roselle were floated on water with Easter lily pollen and allowed to remain from 24 to 48 hours, on examination with the microscope it could be plainly seen that in many places the middle lamellae of the cells had been completely digested. Permanent slides were prepared.

Tests for Bacteria and Molds on Pollen

1. One method was as follows: Extracts of nine different pollens were made, 50 mg. in 10 cc. of water. This extract was then diluted by the usual milk-testing method in sterile bottles to dilutions of 1 : 100 and 1 : 10,000. 1 cc. of this dilution was then placed in sterile petri dishes and agar plates were poured. The plates were then incubated at 37° for 24 hours, in an inverted position to prevent moisture from washing off cultures,

and the colonies were counted. Results from two of several tests are shown, giving the averages (see table 18).

2. 100 mg. of Easter lily pollen was ground well with sterile sand, then well shaken with 100 cc. of 0.85 percent NaCl and 100 mg. of thymol. 1 cc. of this suspension was diluted with 100 cc. sterilized water. Plates of agar were poured with 1 cc. of this dilution and incubated 24 hours at 30°. The average number of colonies on 4 plates was 3. The experiment was repeated, omitting thymol, and the average number on 4 plates was 12. Similar tests with corn, ragweed, and rye gave corresponding results. Thymol does exert an inhibiting action but does not prevent growth of bacteria introduced with the pollen.

3. One gram each of corn, ragweed, rye, and Easter lily pollen was ground with sterile sand and extracted 24 hours in the ice chest with 100 cc. of 0.85 percent NaCl. This solution was filtered through a small, thoroughly sterilized Berkefeld filter into a sterile side-neck flask. Care was taken to plug the side-neck with cotton before sterilizing so that no contamination could enter while filtering by suction. 1-cc. portions of each filtrate were removed with a sterile pipette and agar plates were poured, 4 of each kind of pollen. These were incubated at 37° for 48 hours. No colonies appeared. All the plates were sterile. Six days later one plate had a colony of mold, but this could easily have been later contamination. The sterile filtered extracts were then used for testing for diastase, liquefaction of gelatin, and digestion of fibrin. The diastatic action seemed as rapid as before filtration, but the gelatin liquefaction and fibrin digestion were decreased. The gelatin was completely liquefied after 48 hours, and the rye and ragweed extracts caused only slight digestion of the fibrin. Repetition with other samples confirmed the belief that the filter absorbed the enzyme to a considerable extent. If one could use separate filters for each kind of pollen and work with large quantities, this difficulty might be overcome by allowing the filter to become saturated with the extract.

TABLE 18. *Bacteria and Mold Colony Counts. Averages of Four Plates each. Dilutions 1 : 100 and 1 : 10,000. 37°, 24 Hours.*

Kind of Pollen	Unheated		Autoclaved	
	1 : 100	1 : 10,000	1 : 100	1 : 10,000
Corn	8	0	0	0
Daisy	17	0	0	0
Dock	72	2	14	1
Goldenrod	20	0	0	0
Lily, Easter	2	0	0	0
Pine, Austrian	11	0	0	0
Ragweed	43	1	0	0
Rye	31	2	0	0
Timothy	27	14	0	0

4. Washing the pollen before use was tried with corn, ragweed, and Easter lily. 1 gram of unground pollen was shaken vigorously with 300 cc. of distilled water in a liter cylinder, and then filtered into a sterile flask through a sterilized Buchner funnel and filter paper, and washed four times with sterile distilled water. Plates were poured from the last washing, dilution 1 : 100. Some plates were sterile and others from the same filtrate had from 1 to 19 colonies. The washed pollen when diluted and tested showed a similar lack of constancy, so that there seems to be no gain in washing, and this method may cause loss of enzymes.

Repetition with dock pollen failed to show that it always had a higher count. There was probably some initial contamination in preparing the extract.

Summary

Although it has been assumed that pollen tubes digest their way through the style, there is little experimental evidence as to the exact nature of this enzyme action. Histological examination shows that in most instances pollen tubes make their way between the walls of adjacent cells rather than penetrating them. We should expect therefore to find most frequently not a cytase- or cellulose-digesting enzyme, but rather a pectinase capable of digesting the pectin of the inner lamella. This has been proved in the writer's experiments to be the case.

Eighteen species of pollen have been used; these have been tested for thirteen kinds of enzymes. So far amylase, invertase, catalase, reductase, and pectinase have been found in all. Pepsin, trypsin, erepsin, and lipase have been demonstrated in some and not in others. Cytase was doubtfully identified in six of the eighteen. Tyrosinase and laccase have not been found in any, and zymase was found only in Siberian crab apple pollen.

BIBLIOGRAPHY

Adams, J. 1916. On the germination of the pollen grains of apple and other fruit trees. Bot. Gaz. **61**: 131–147.

Allen, C. E. 1901. On the origin and nature of the inner lamella. Bot. Gaz. **32**: 1–34.

Amici, G. B. 1830. Note sur la mode d'action du pollen sur le stigmate. Ann. Sci. Nat. **21**: 329–332.

Biourge, P. 1892. Recherches morphologiques et chimiques sur les graines de pollen. La Cellule **8**: 47–76.

Bower, F. O. 1919. Botany of the living plant. London.

Braconnot, H. 1829. Recherches chimiques sur le pollen du *Typha latifolia*. Ann. Chim. Phys. **42**: 91–105.

Church, A. H. 1875. Some contributions to plant chemistry. Jour. Bot. **13**: 169.

Coulter, J. M. 1910. Morphology of the gymnosperms. Chicago.

——, and Chamberlain, C. J. 1903. Morphology of the angiosperms. New York.

Crabill, C. H., and Reed, H. S. 1915. Convenient methods for demonstrating the biochemical activity of micro-organisms, with special reference to the production and activity of enzymes. Paper no. 29, Lab. Plant Path. Bact., Va. Agr. Exp. Sta.

Czapek, F. 1905. Biochemie der Pflanzen. Jena.

——. 1913–1921. Biochemie der Pflanzen [2nd. ed.]. Jena.

Elfving, F. 1879. Studien über die Pollenkörner der Angiospermen. Jena. Zeitschr. Naturwiss. **13**: 1–28.

Erlenmeyer, E. 1874. Über die Fermente in den Bienen, im Bienenbrot und im Pollen, etc. München Akad. Sitzungsber. math.—phys. cl. **4**: 204–207.

Frémy, E. 1848. Untersuchung über das Reifen der Früchte. Ann. der Chem. und Pharm. **67**: 332.

Goodale, J. L. 1916. Pollen therapy in hay fever. Ann. Otol. Rhinol. Laryngol. **24**: 269–285.

Gow, J. 1907. Morphology of *Spathyema foetida*. Bot. Gaz. **43**: 131–136.

Green, J. R. 1894. Researches on the germination of the pollen grains and the nutrition of the pollen tubes. Phil. Trans. Roy. Soc. London B **185**^{1}: 385–409.

Guignard, L. 1886. Sur la pollinisation et ses effets chez les Orchidées. Ann. Sci. Nat. Bot. VII, **4**: 202–240.

Hansgirg, A. 1897. Beiträge zur Biologie und Morphologie des Pollens. Sitzungsber. Ges. Wiss. Prag. **13**^{1}: 1–76.

Heyl, F. W. 1917. Analysis of ragweed pollen. Jour. Amer. Chem. Soc. **39**^{2}: 1470–1476.

——. 1919 *a*. The protein extract of ragweed pollen. Jour. Amer. Chem. Soc. **41**: 670–682.

——. 1919 *b*. The yellow coloring substances of ragweed pollen. Jour. Amer. Chem. Soc. **41**: 1285–1289.

Jost, L. 1905. Zur Physiologie des Pollens. Ber. Deutsch. Bot. Ges. **23**: 504–515.

Kammann, O. 1904. Zur Kenntniss des Roggen-Pollens und des darin enhaltenen Heufiebergiftes. Beitr. Chem. Physiol. Path. **5**: 346–354.

Kerner, A. J. 1895. The natural history of plants. Translated by Oliver. New York.

Kirkwood, J. E. 1906. The pollen tube in some of the Cucurbitaceae. Bull. Torrey Club **33**: 327–342.

Knight, L. J. 1918. Physiological aspects of self-sterility of the apple. Proc. Amer. Soc. Hortic. Sci. **14**: 101–105. See Bot. Absts. **1**: Entry 964.

Koessler, J. H. 1918. Studies on pollen and pollen disease. I. The chemical composition of ragweed pollen. Jour. Biol. Chem. **35**: 415–424.

Land, W. J. G. 1907. Fertilization and embryogeny in *Ephedra trifurca*. Bot. Gaz. **44**: 273–292.

Lidforss, B. 1899. Weitere Beiträge zur Biologie des Pollens. Jahrb. Wiss. Bot. **33**: 232–312.

Liefmann, H. 1904. Ein Beitrag zur Frage nach der ätiologischen Bedeutung gewisser Pflanzen-Pollenkörner für das Heufieber. Zeitschr. Hygiene **47**: 153–178.

Mangin, L. 1888. Sur la constitution de la membrane des végétaux. Compt. Rend. Acad. Sci. Paris **107**: 144.

——. 1889. Sur la presence des composés pectique dans les végétaux. Compt. Rend. Acad. Sci. Paris **109**: 579.

——. 1893. Sur l'emploi du rouge de ruthénium en anatomie végétale. Compt. Rend. Acad. Sci. Paris **116**: 653.

Miller, E. 1919. Development of the pistillate spikelet and fertilization in *Zea Mays* L. Jour. Agr. Res. **18**: 255–265.

Molisch, H. 1893. Zur Physiologie des Pollens mit besonderer Rücksicht auf die chemotropischen Bewegungen der Pollenschläuche. Sitzungsber. Akad. Wien, Math.-Naturw. Cl. **102**^{1}: 423–448.

Mottier, D. M. 1904. Fecundation in plants. Carnegie Inst. Pub. Washington.

Müller, H. 1883. Arbeitsteilung bei Staubgefässen von Pollenblumen. Kosmos **13**: 241–259.

Planta, M. 1885. Landwirthsch. Versuchsstaat. **32**: 215–230.

Plimmer, H. G. 1912. Color of pollen. Lancet **2**: 51.

Przybytek, A., and Famintzin, A. 1886. Über die chemische Zusammensetzung der Blüthenstaubasche der Kiefer (*Pinus sylvestris*). Reviewed in Ber. Chem. Ges. **19**^{3}: 32.

Rittinghaus, P. 1886. Beobachtungen über das Eindringen der Pollenschläuche ins Leitgewebe. Verh. Nat. Ver. Rheinland **43**: 105–122.

Sandsten, E. P. 1909. Some conditions which influence the germination and fertility of pollen. Univ. Wis. Agr. Exp. Sta. Res. Bull. 4. 1909.

Sawyer, M. L. 1917. Pollen tube and spermatogenesis in Iris. Bot. Gaz. **64**: 159–164.

Schleiden, M. J. 1849. Principles of botany. Transl. by E. Lankester. London.

Shreve, F. 1906. The development and anatomy of *Sarracenia purpurea*. Bot. Gaz. **42**: 107–115.

Smith, G. 1904. Plant mucilages. Jour. Soc. Chem. Ind. **25**: 105, 972.

Stift, A. 1896. Über die chemische Zusammensetzung des Blütenstaubes der Runkelrübe. Reviewed in Bot. Centralbl. **65**: 43, 44.

——. 1901. Über die chemische Zusammensetzung des Blütenstaubes der Zuckerrübe. Bot. Centralbl. **88**: 105–107.

Stoklasa, J. 1896. Das Lecithin in der Pflanze. Sitzungsber. Akad. Wiss. Wien. **105**^{1}: 604–632.

Strasburger, E. 1886. Über fremdartige Bestäubung. Jahrb. Wiss. Bot. **17**: 50–98.

Tischler, G. 1910. Untersuchungen über den Stärkegehalt des Pollens tropischer Gewächse. Jahrb. Wiss. Bot. **47**: 219–242.

Ulrich, H. L. 1914. Some notes on hay fever. Jour. Amer. Med. Assoc. **62**: 1220–1222.

Van Tieghem, P. 1869. Recherches physiologiques sur la végétation libre du pollen et de l'ovule et sur la fécondation directe des plantes. Ann. Sci. Nat. Bot. V, **12**: 312–328.

Wiesner, J. von. 1914. Die Rohstoffe des Pflanzenreiches. Leipzig.

Wodehouse, R. P. 1916. A simple method of obtaining ragweed pollen in large quantities. Boston Med. Surg. Jour. **174**: 430.

THE EMBRYOGENY OF CYRTANTHUS PARVIFLORUS BAKER

Wm. Randolph Taylor

(Received for publication April 7, 1921)

Introduction

The question of the relationship of monocotyledonous and dicotyledonous plants, long and hotly debated and yet unsettled, has of late years largely been attacked from two angles, the sequence and position of organ formation in the early developing embryo, and the vascularization of the seedling. The origin of the monocotyledonous type from the primitive dicotyledons at one or more points seems an established probability, but the stages of the transfer from the one into the other have not been completely filled out, and apparent anomalies will remain until we have knowledge of a vastly greater series of forms than at present.

In 1917 the writer began the preservation and sectioning of material of *Cyrtanthus parviflorus* Baker, with the principal object of securing a complete series of embryo-sac and embryo stages for demonstration purposes. The work had proceeded for some time before he came upon the paper of Miss Farrell on the related *Cyrtanthus sanguineus* (Lindl.) Hook., when it was at once evident that the embryological history in the two forms is vastly different. From time to time more material of *Cyrtanthus parviflorus* was prepared, until at present a practically unbroken series of stages has been secured from fertilization to seed maturity. Young seedlings have also been sectioned to follow the history of the vascular bundles and other structures in germination. Although this species does not present any new situation in monocotyledonous embryogeny, the completeness of the material and the variation it would seem to indicate within a single genus will perhaps warrant the presentation of this description as a little evidence toward a solution of the major problem.

Material and Observations

Under greenhouse culture the plants practically never set seed unless hand-pollinated; this rendered unnecessary the castration and bagging of the flowers. Fixations followed at suitable intervals after pollination, and full records of dates and of time elapsed were preserved. About fifty post-fertilization fixations were made and studied, in addition to a lesser number dealing with the maturation of the pollen and of the embryo sac.

Fertilization follows pollination in from 36 to 50 hours, seeming to vary somewhat in the different batches. The egg is enclosed in a definite membrane at the micropylar end of the sac and is fertilized in that position;

the polar nuclei have united during the maturation of the embryo sac and have passed to the antipodal end where the triple fusion occurs. Both zygote and primary endosperm nuclei then remain in a state of rest with finely divided extra-nucleolar chromatin for some time. Three or four successive divisions of the endosperm nucleus precede the first division of the zygote, which occurs in from 94 to 118 hours after pollination. The first and second endosperm mitoses occur close to the antipodals at the base of the embryo sac, but the nuclei produced soon move down along the sides of the sac and with subsequent free nuclear divisions become equally distributed in the peripheral cytoplasm. The sac is large and increases in size rapidly after fertilization, so that there seems to be no evidence of pressure upon the embryo until centripetal wall formation occurs in the endosperm, after all parts of the embryo have become well established (4, p. 433).

The first divisions of the zygote give rise to a filament of three, occasionally four, cells (figs. 1, 2, 3). Then transverse divisions and vertical walls occur in the terminal and subterminal cells of the row (fig. 4). The lowest cell is primarily concerned in the formation of the suspensor, which is unicellular or divided at the top by one to three more or less oblique walls (figs. 5, 9). The embryo proper develops as an ovoid mass until it has attained a length of 8–10 cells, when it becomes evident that the structure is no longer radially symmetrical, but that it has developed a bilateral symmetry, one side being more convex than the other (figs. 5, 6, 7, 8). All the tissue beyond the point of indentation becomes the cotyledon, which seems to be derived from the end cell of the primitive row. It seems equally clear that the root is derived from the middle cell, but the conditions in the region of the growing point are not so certain. The swelling on the lower portion of the indentation gives rise first to an outer ridge that connects with the cotyledon on the sides, and subsequently, within and protected by this ridge, to a series of 2 or 3 plumular leaves (figs. 9, 10, 11).

The first sign of the development of the vascular system appears at the same time as the first plumular leaf, in the form of a pair of bundles in the cotyledon (fig. 13). These soon join with the rudiment of the axial system at the cotyledonary node. In the old embryo this node has four principal poles, two connected with the bundles which traverse the cotyledon, two with bundles which run forward and curve up, joining with the main cotyledonary traces above the tip of the plumule. These loops first appear near their attachment at the node, and subsequently effect union with the main trace (figs. 16, 17, 19). The rudiment of a single bundle appears in the older plumular leaf before germination (figs. 18, 20). As the size of the embryo increases, the ridge early formed connecting the edges of the cotyledon elongates and widens, forming with the cotyledon a short sheath around the plumule. The base of the cotyledon becomes quite broad and curved so that the looped bundle traces appear in a transverse section to lie opposite, or even a little in front of, the edges of the first leaf (fig. 23). With germination the basal sheathing portion of the cotyledon containing the vascular loops elongates greatly, so that the foliage leaves issue from a long tubular cotyledonary sheath (figs. 22, 24, 25).

Discussion

The first question which arises in the consideration of plants, for which there is reported so different a history of a conservative structure, is the degree of relationship of the forms. *Cyrtanthus sanguineus* (Lindl.) Hook. was the first described (6). It belongs to the subgenus Gastronema, which by some has been considered as of generic rank. However, the points that distinguish this subgenus are but slight and quantitative rather than qualitative, the flowers being fewer, larger, and more erect than in the other subgenera. *Cyrtanthus parviflorus* was described by Baker in 1891 (1). In the *Flora Capensis* of Thiselton-Dyer he places it in the subgenus Monella which differs from the subgenus Cyrtanthus chiefly in having linear rather than lorate leaves (9). There is little from the systematic standpoint, therefore, to indicate that the forms are not closely related.

The first paper touching upon the embryo of *Cyrtanthus sanguineus* was that of Miss Farrell in May, 1914 (4). In June of the same year Coulter and Land gave some additional details (2). The condition in *Cyrtanthus sanguineus* as interpreted by these writers is, briefly, as follows: At the top of the ovoid proembryo arise four swellings or growing points. These are soon followed by a ring of growth upon which they are raised up as upon a tube. Then they merge in twos by an active growth of the tissue between the pairs. Subsequently one duplex structure greatly outgrows the other, forming the prominent cotyledon, while the other, which does not shoot up, with the tubular portion constitutes the sheath. For her paper, Miss Farrell had available but two stages of embryo material, one showing the tubular stage with the swellings on the rim, the other a nearly mature embryo. This would seem a very small amount on which to base the important conclusions at which she arrives. Coulter and Land appear to have had additional material, though they do not figure it. As a result of their observations on embryos, Coulter and Land conclude in part as follows:

> In the embryogeny of both monocotyledons and dicotyledons, a peripheral cotyledonary zone gives rise to two or more growing points, or primordia; this is followed by zonal development, resulting in a cotyledonary ring or sheath of varying length. If both growing points continue to develop equally, the dicotyledonous condition is attained; if one of the growing points ceases to develop, the continued growth of the whole cotyledonary zone is associated with that of the other growing point, and the monocotyledonous condition is attained. In like manner, polycotyledony is simply the appearance and continued development of more than two growing points on the cotyledonary ring. It follows that cotyledons are always lateral structures, arising from the peripheral zone developed at the top of the more or less massive proembryo (3).

In *Cyrtanthus parviflorus* it seems fairly clear to the writer that the cotyledon originates now as a terminal structure, no matter what, evolutionarily, its status may have been. The growing point forms well down on one side of the embryo, its location presaged by the smaller size of the cells and their denser protoplasmic contents before indentation becomes evident. As a semilunar ridge on the lower side of the meristematic region there arises a sheath which, joined with the base of the cotyledon, functions as do the sheathing bases of many other monocotyledonous leaves. The mature stage of *Cyrtanthus sanguineus* agrees closely with that of *Cyrtanthus parviflorus*. The writer would consider that the species he has studied corresponds through its early stages in all essentials with the familiar cases of Alisma (5) and Sagittaria (8), and that it does not lend support to the view of Coulter and Land quoted above.

With regard to the general question of the origin of the monocotyledonous type, this form, as an isolated case, can add little. Its vascular anatomy is easily connected with that of other Amaryllidaceous types previously described (7), but gives no decisive evidence for or against the theory of the origin of the single cotyledon by the edge-to-edge union of the two dicotyledonous seed leaves. Worsdell seems to consider that the condition in *Cyrtanthus sanguineus* is an abnormal one, an example of a minor instance of progressive evolution (10). Whether it is an isolated case, or connected with the more ordinary *Cyrtanthus parviflorus* by intermediate stages, it remains for a wider study of the genus to demonstrate.

University of Pennsylvania

BIBLIOGRAPHY

1. **Baker, J. G.** *Cyrtanthus* (*Nemella*) *parviflorus* n. sp. Gardener's Chronicle III, **9**: 104. 1891.
2. **Coulter, J. M., and Land, W. J. G.** The origin of monocotyledony. Bot. Gaz. **57**: 509–519. 1914.
3. ——. The origin of monocotyledony II. Monocotyledony in grasses. Ann. Mo. Bot. Gard. **2**: 175–183. 1915.
4. **Farrell, M. E.** The ovary and embryo of *Cyrtanthus sanguineus*. Bot. Gaz. **57**: 428–436. 1914.
5. **Hanstein, J.** Die Entwickelung des Keimes der Monocotylen und Dikotylen. Bot. Abhandl. Bonn **1**^{1}: 1–112. 1870.
6. **Lindley, J.** New plants, etc., from the Society's garden, no. 32. *Gastronema sanguineum*. Jour. Hort. Soc. London **3**: 315–316. 1848.
7. **Sargant, E.** A theory of the origin of monocotyledons, founded on the structure of their seedlings. Annals of Bot. **17**: 1–92. 1903.
8. **Schaffner, J. H.** Contribution to the life history of *Sagittaria variabilis*. Bot. Gaz. **23**: 252–273. 1897.
9. **Thiselton-Dyer, W. T.** Flora Capensis (Amaryllidaceae by J. G. Baker) **6**: 218–228. 1896–1897.
10. **Worsdell, W. C.** The morphology of the monocotyledonous embryo and that of the grass in particular. Annals of Bot. **30**: 509–524. 1916.

DESCRIPTION OF FIGURES

PLATE XXV

FIGS. 1–4. Fertilized egg and filamentous stage of the proembryo. × 150. Figure 1, 65 hours after pollination; figures 2, 3, 332 hours, figure 4, 284 hours after pollination.

FIGS. 5–8. Development of the bilaterally symmetrical embryo. × 150. Sagittal sections. Figure 5, 294 hours after pollination; figure 6, 428 hours, figure 7, 480 hours, figure 8, 600 hours after pollination.

FIGS. 9–10. Formation of the lateral growing point, sagittal sections. × 150. Figure 9, 480 hours, figure 10, 600 hours after pollination.

FIGS. 11–12. Origin of the sheath and of the first leaf, sagittal sections. × 75. Figure 11, 576 hours, figure 12, 648 hours after pollination.

PLATE XXVI

FIGS. 13–20. Stages in the development of the vascular strands and of sheath and leaf. Figures 13, 19, frontal sections; figures 14, 15, 17, 18, 20, sagittal; figure 16, intermediate. × 30. Figures 13, 14, 648 hours, figure 15, 576 hours after pollination; figure 16, age unknown; figure 17, 762 hours, figures 18, 19, 20, 888 hours after pollination. All are reconstructions from several serial sections. Figure 16 shows a case where one of the loops has failed to develop. The partial suppression of one loop was observed on several occasions.

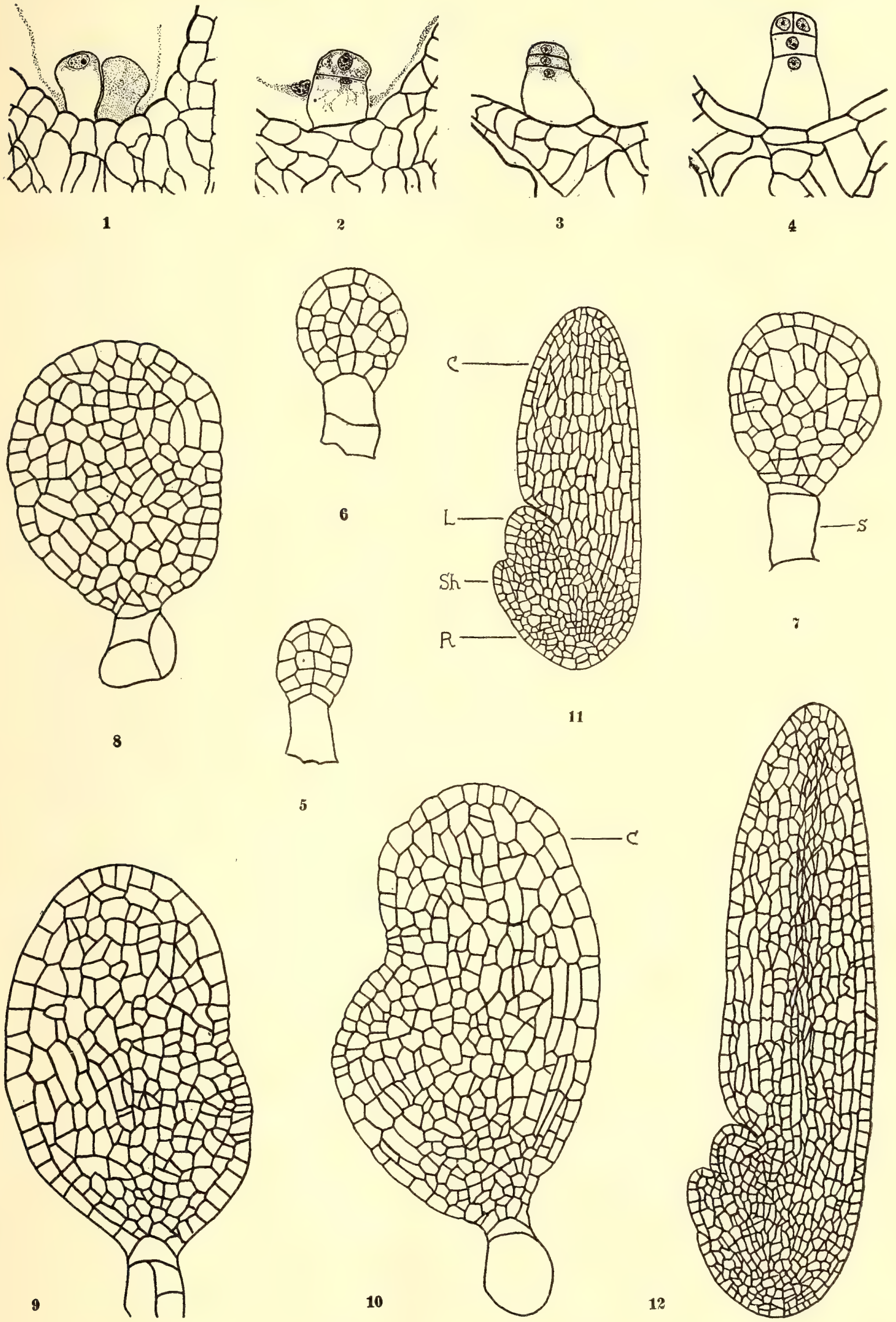
FIG. 21. Surface view of embryo showing the sheath and the first leaf. The shapes of the cells at the margin of the sheath and in the leaf and surrounding portion of the cotyledon have been indicated. × 90.

FIG. 22. Front view of sheath. The cotyledon was split sagittally from the rear and the sheathing base was spread out on the slide. All cut edges indicated by broken lines; vascular bundles by dotted lines. × 9.

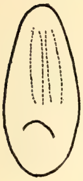
FIG. 23. Transverse section of cotyledon and sheath. This shows the positions of the vascular bundles and of the first leaf. × 75.

FIGS. 24, 25. Seedlings, about two weeks after germination. These show the length of the sheath. In figure 24 the seed has been entirely removed from the absorbing cotyledon, but in figure 25, although the seed coats have been removed, the endosperm is intact. Vascular bundles indicated by dotted lines. × 3.

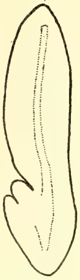
"*S*" suspensor, "*R*" radicle, "*Sh*" sheath, "*L*" first leaf, "*C*" cotyledon, "*E*" endosperm.


1
2
3
4
6
C
L
Sh
R
7
S
8
11
5
C
9
10
12

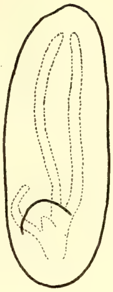
TAYLOR: EMBRYOGENY OF CYRTANTHUS PARVIFLORUS.



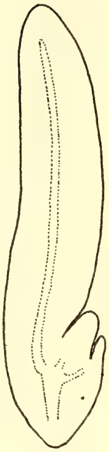
13



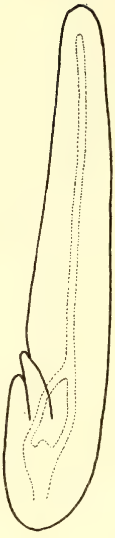
15



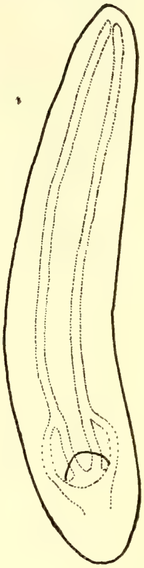
16



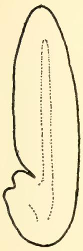
17



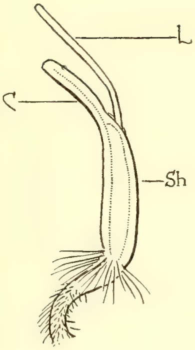
18



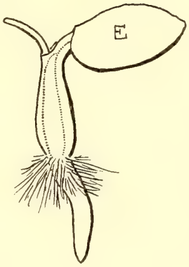
19



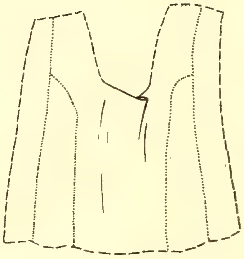
14



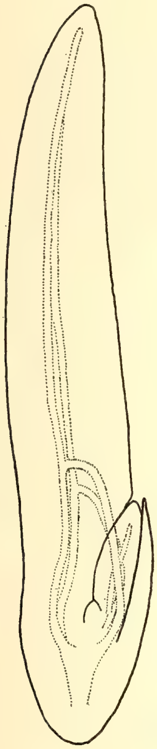
24



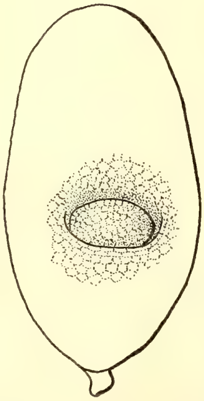
25



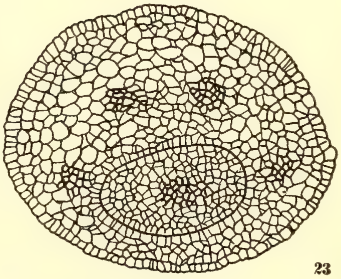
22



20



21



23

TAYLOR: EMBRYOGENY OF CYRTANTHUS PARVIFLORUS.

STUDIES ON PLANT CANCERS III. THE NATURE OF THE SOIL AS A DETERMINING FACTOR IN THE HEALTH OF THE BEET, BETA VULGARIS, AND ITS RELATION TO THE SIZE AND WEIGHT OF THE CROWN GALL PRODUCED BY INOCULATION WITH BACTERIUM TUMEFACIENS[1]

Michael Levine

(Received for publication April 16, 1921)

Little is known concerning either the effect on the size and the weight of beets, either spontaneously or artificially infected with crown gall, or the effect of the general vigor of the plant on its susceptibility to infection. Many problems of practical and theoretical importance along these lines remain to be solved. Among them are: (1) a more accurate evaluation of the effects of the crown-gall disease upon the final size of the infected beet and hence on the beet crop; (2) the cause of the greater susceptibility of the sugar beet as compared with other races to the crown-gall organism; (3) the breeding of beets to obtain strains immune to crown-gall infection and yet retaining the desirable marketable qualities; (4) the cytological difference between crown gall and "tuberculosis" of beets and the relation of the former to animal cancer; (5) the relation of the soil to the health of the beet and to the size of the crown gall that it will harbor when inoculated with *Bacterium tumefaciens*.

Up to the present very little evidence has been advanced by animal pathologists to show any definite relation between the physical condition and the virulence of cancer once it is started.

The purpose of this paper is to present some data on the last of the questions noted above; namely, the relation of the health of the beet to the size of the crown gall resulting from infection. Since the term "health" has but a relative meaning, we shall measure health in this case in terms of size and weight, the matters most important to the beet grower and without doubt fair indices of the vigor and normality of the beet plant.

Inasmuch as crown gall is analogous to animal cancer, as Smith (1911, 1912), Magnus (1918), Levine (1919, 1920), Levin and Levine (1920), and Jensen (1918) have pointed out, these results also have a bearing on the general question of feeding in cancer and a more specific relation to the question of the relation of general vigor and vitality to susceptibility.

The older literature concerning the crown gall of beets is too well known to need mention here. Only those papers that deal with the problem at hand are reviewed.

---

[1] From the Department of Cancer Research of Montefiore Hospital, Dr. I. Levin, Chief.

Tumor-like growths on the different varieties of beets have been recognized in America and Europe for a long time. These growths occur sporadically. Destructive epidemics are not known. Smith, Brown, and Townsend (1911) have shown that at least the gall formation known as crown gall[2] of the beet is produced by *Bacterium tumefaciens.* They were able to isolate the organism from the tumors on the beet and to infect beets, causing the formation of new crown galls.

Townsend (1915), studying the crown gall of the sugar beet which appears spontaneously in the field, concluded that the galls have no marked effect upon the size of the beet. The largest as well as the smallest beets may become seriously affected, and it is impossible to know whether roots would be large or small if they had been free from the infection. The galls begin to appear when the beets are one fourth to one half grown, that is, about midsummer; and from that time on they may appear at any time until harvest, so that crown galls of various sizes and ages may be found on the beets.

Jensen (1918) in his investigation of tumor-like growths in plants studied both spontaneous crown galls from the common races of beets and those that he produced experimentally by inoculation. He concludes that in the case of the mangel wurzel the crown gall has no detrimental effect upon the growth of the plant. In the garden beet, the tumor attains only slight size. In the sugar beet, on the other hand, Jensen finds the disease resulting in enormous tumor-like formations, irregular knotty structures which in section exhibit a very irregular arrangement of the vascular bundles differing markedly from that in the normal portion of the root. Jensen does not report on the relative sizes of the galls and roots in the sugar beet, nor does he discuss the structural characters of the normal roots of the various races for possible suggestions as to the cause of the differences in the size and structure of the galls.

Materials and Methods

The cultivated garden varieties, Early Model, Egyptian Early, and Giant Mangel Wurzel of *Beta vulgaris* were studied in my experiments. A preliminary test of the effect of the soil on the size of the root and crown gall produced when the roots were inoculated with *Bacterium tumefaciens* was carried out in six-inch pots in which a mixture of Early Model and Egyptian Early seeds was planted. Four kinds of soil were used. Each kind was placed in 12 pots. The first group was filled with garden soil mixed with an abundance of manure that had been previously used for mushroom culture. The second contained a brown loam that was obtained from a neighboring lot, and the third, a combination of equal parts of garden soil and manure and of the loam used in the second. The fourth group was filled with a medium sand used for building purposes. Three seedlings were planted in each pot. The pots were placed in boxes, and the spaces between the pots were filled with hay. The boxes were then placed in the open where they were exposed to the light all day. When the tap roots began to appear, the soil was gently removed from one side of each root, and, with a needle dipped in a culture of *Bacterium tumefaciens*, the root was pricked five to fifteen times. The soil was then returned and the plants were not disturbed again until the middle of October. Then they were carefully removed from the soil and studied. In each group of pots, some plants were left uninoculated to serve as controls.

---

[2] "Tuberculosis" of beets is a tumor-like growth on beets produced by *Bacterium beticolum* (Smith, Brown, and Townsend, 1911).

Field studies were made in three plots 12 x 25 feet each. The first two, "E" and "W," consisted of soil well worked and thoroughly mixed with an abundance of manure. The third, "SW," consisted of a coarse sandy soil never used before and unfertilized. In early May, the plots "E" and "SW" were planted with a mixture of Early Model and Egyptian Early. The rows were thinned out in June, and in the latter part of July, when the tap roots began to appear, they were inoculated in the manner described above with young cultures of *Bacterium tumefaciens*. Each inoculated plant was labeled. An equally large number of uninoculated plants were left growing among the inoculated ones to serve as controls. The plots were equally well exposed to sunlight for the greater part of the day. They were similarly worked and watered. A considerable number of inoculated plants were gathered from time to time and fixed for a cytological study to be reported on at a later time.

Plot "W" was sown with the Giant Mangel Wurzel seeds and treated as the plots "E" and "SW" were.

Relation of the Soil Quality to the Size and Weight of the Root and of the Crown Gall

We may consider first the experiments in which the beets were grown in pots as described above. The effect of the crown gall on the total beet crop grown under these conditions is only suggestive since the number of our experiments was relatively small. The relation of the size and weight of the root to the size and weight of the crown gall is the point with which we are specially concerned.

In this series of experiments, as noted, a mixture of seeds of the garden beet varieties, Early Model and Egyptian Early, were grown in 48 six-inch pots in four different kinds of soil. In the first group, made up of 12 pots, the soil consisted of a mixture of manure that had previously been used for mushroom culture and an equal quantity of garden soil. The second group of pots were filled with a brown silt loam, the third group contained equal parts of manure and brown silt loam, and the fourth contained a medium sand.

TABLE 1. *Effect of the soil on the weights*[3] *of the garden beet, Beta vulgaris, vars. Early Model and Egyptian Early, the roots of which were inoculated with Bacterium tumefaciens. Growing in pots containing "A" garden soil and manure, "B" brown silt loam, "C" manure and brown silt loam, "D" medium sand*

No.	Pot A		Pot B		Pot C		Pot D	
	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry
1......	31.75	3.30	81.40	5.40	38.30	3.00	36.80	3.60
2......	60.00	5.60	46.00	4.50	2.10	1.50	30.00	3.80
3......	113.60	11.80	37.10	3.00	46.10	4.70	38.60	3.60
4......	65.40	5.50	24.60	1.80	32.10	3.30	33.50	4.50
5......	38.60	4.60	51.00	4.10	10.00	1.20	23.70	2.70
6......	45.50	4.80	24.10	2.30	22.30	2.10	36.00	4.20
7......	45.00	5.30	26.00	2.70	36.50	4.40	37.70	4.30
8......	42.50	4.60	33.10	3.00	22.60	2.40	5.10	.90
9......	67.50	5.30	19.10	1.40	5.70	1.20	8.70	.90
10......	51.10	6.15	60.00	3.90	13.20	1.60	53.70	5.50
11......	71.10	7.10	66.60	7.50	9.50	1.00	13.70	1.90
12......	78.10	9.00	21.60	1.20	7.10	.80	9.00	2.10
13......	49.60	4.50	17.00	1.50	32.50	2.80	14.90	2.30
14......	25.50	2.20	9.50	1.60	51.00	5.00	24.00	2.10
15......	13.20	1.80	34.00	3.50	74.00	8.40	20.00	2.60
								corrections
16......	33.90	3.10	19.00		66.70	7.80	33.00	.80
17......	36.30	3.60	22.50	1.70	22.50	2.20	24.10	5.50
18......	22.40	2.50	27.50	2.60	55.80	4.40	3.70	3.60
19......	13.80	1.30	10.30	corrections	12.40	1.80	6.00	
20......					31.00	3.15		
21......					27.80	2.90		
22......				2.60	51.20	5.50		
23......				8.60	16.00	1.80		
24......				4.50	13.40	1.70		
25......				2.70	10.00	1.00		
Totals...	904.85	92.05	630.40	63.00	728.70	75.65	452.20	53.50
Averages	46.57	4.73	33.17	3.316	29.14	3.02	23.80	2.81

The plants, three to four in each pot, were inoculated as described above with *Bacterium tumefaciens* on July 20, 1920. On October 9, 1920, the plants were removed from the soil, and the sizes and weights of the roots grown in the different soils were compared.

Figure 1 shows the general development of the plants as they appeared in the pots and represents two average pots from each group of twelve, of the varieties Early Model and Egyptian Early. "A" represents the plants grown in the garden soil with an abundance of manure, "B" the plants in the brown silt loam, "C" the plants in brown silt loam and manure, and "D" the plants grown in medium sand.

The plants grown in pot "A" (garden soil plus manure) are much larger than those in pots "C" (brown silt loam and manure) and in "D" (sand), but the difference is not so marked between "A" (garden soil and manure) and "B" (brown silt loam). The leaves of the plants growing in pot "A" are somewhat larger and appear to be more numerous. While we

[3] Weights given in grams in all tables.

have no very reliable basis for measuring health or vitality quantitatively, it would be generally conceded that plants such as those represented in pot "A" are more vigorous and more healthy than those in pot "D."

On removing the plants from the soil, it was found that the size of the leaves generally served as a good indication of the size of the roots. The roots whether inoculated or uninoculated were largest when grown in the soil and manure, smaller when grown in brown silt loam or in the combination of the loam and manure, and smallest when grown in sand.

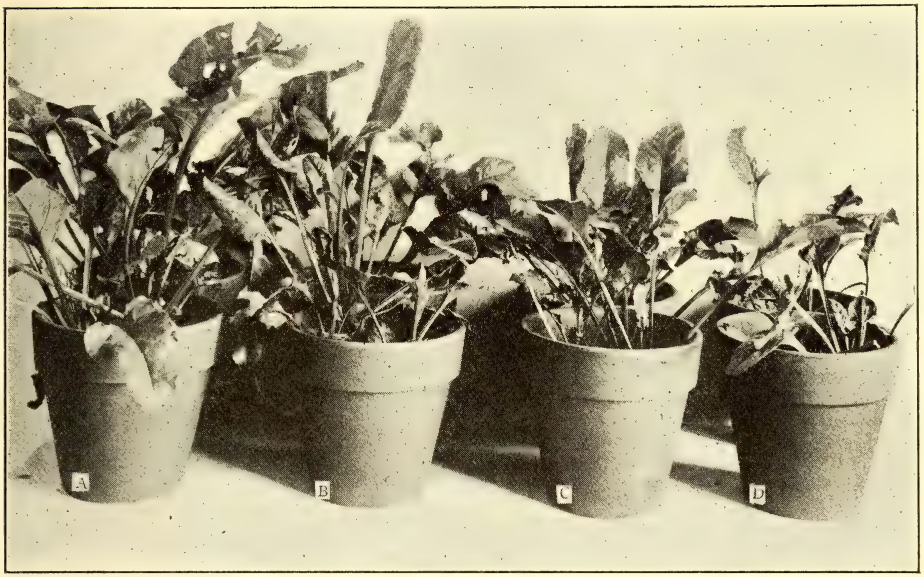


FIG. 1, A, B, C, and D. Four pots filled with manure, brown silt loam, manure and brown silt loam, and sand, respectively.

The size and weight of the crown gall in each case was directly proportional to the size and weight of the root, as will be shown below. It is seen that the weight of the entire plant grown in the four different soils shows that those plants which were grown in soil rich in organic material were well nourished and attained the greatest weights while those grown in sand weighed less. Table 1 gives the fresh and dry weights of the entire plants grown in the four different soils with their roots inoculated with *Bacterium tumefaciens*. Representative plants with their infected roots from each group are shown in figure 2. Figure 2, "A" shows an infected root of a plant grown in garden soil mixed with an abundance of manure. "B" was grown in brown silt loam and manure. "C" was grown in brown silt loam, and "D" was grown in sand. As shown in table 1, the infected plants grown in pots containing garden soil and manure attained a fresh weight of 46.57 g. and a dry weight of 4.73 g., while plants grown in brown silt loam were next highest with an average weight when fresh of 33.17 g. and a dry weight of 3.32 g., and the plants grown in a combination of loam and manure fell slightly short of the weights attained in the loam alone. The difference, however, is slight. A striking difference is seen in the proportionate size of the crown galls on the roots shown in figure 2, "A" and "D," and also in the average weights of the plants which are grown in the garden soil and sand respectively (see table 1). These results are quite striking and indicate rather clearly that the better nourished host responds to the parasitic infection more actively than the poorly nourished one, a fact which is generally recognized in animal pathology.

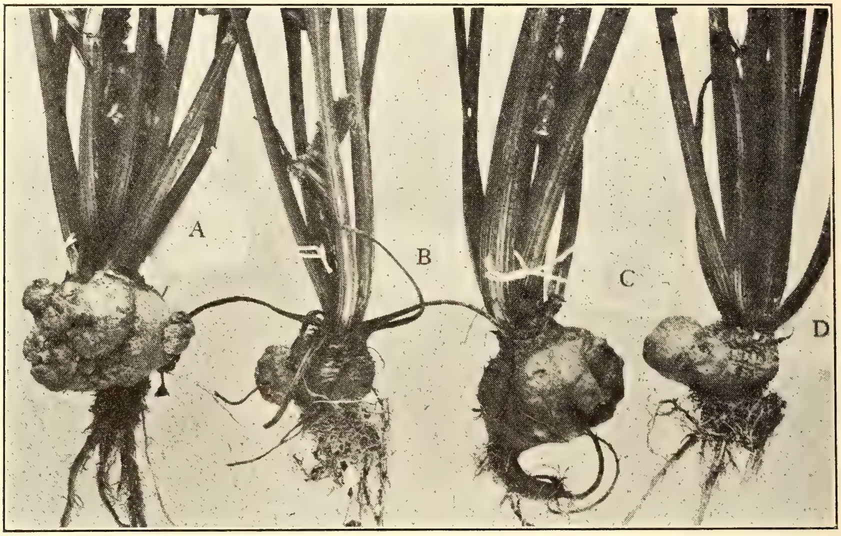


Fig. 2, A, B, C, and D. Roots of beets grown in pots filled with manure, manure and brown silt loam, brown silt loam, and sand respectively.

Better nutrition of the host does not tend to increased resistance to the growth of an inoculum of an animal tumor, but appears rather to have the contrary effect as shown by Ewing. It appears from these experiments that when the host is well fed the parasite is also well fed, it reproduces more actively, and produces a greater quantity of toxin which apparently calls forth relatively a greater hyperplasia of the host. The case is very clear here that normal development of the host rather favors the development of the crown gall. There is no evidence of increased resistance to the parasite in well grown as compared with poorly grown plants.

RESULTS OF THE FIELD EXPERIMENT

As mentioned above, similar seeds of *Beta vulgaris*, varieties Early Model and Egyptian Early, were planted in two plots, "E" and "SW," with an area of 300 sq. ft. each. The first, "E," had been used during three previous seasons for root crops. The soil was well worked and abundantly treated with thoroughly rotted manure. The plot "SW" had never been used for a crop. It consisted of filled-in land. The soil was coarse and made up chiefly of coarse sand. No fertilizer was used on this plot. Beginning August 4 to 15, 1920, three hundred roots in each plot were inoculated with young cultures of *Bacterium tumefaciens* and labeled. A large number of beet roots growing among the inoculated plants were left undisturbed to serve as controls.

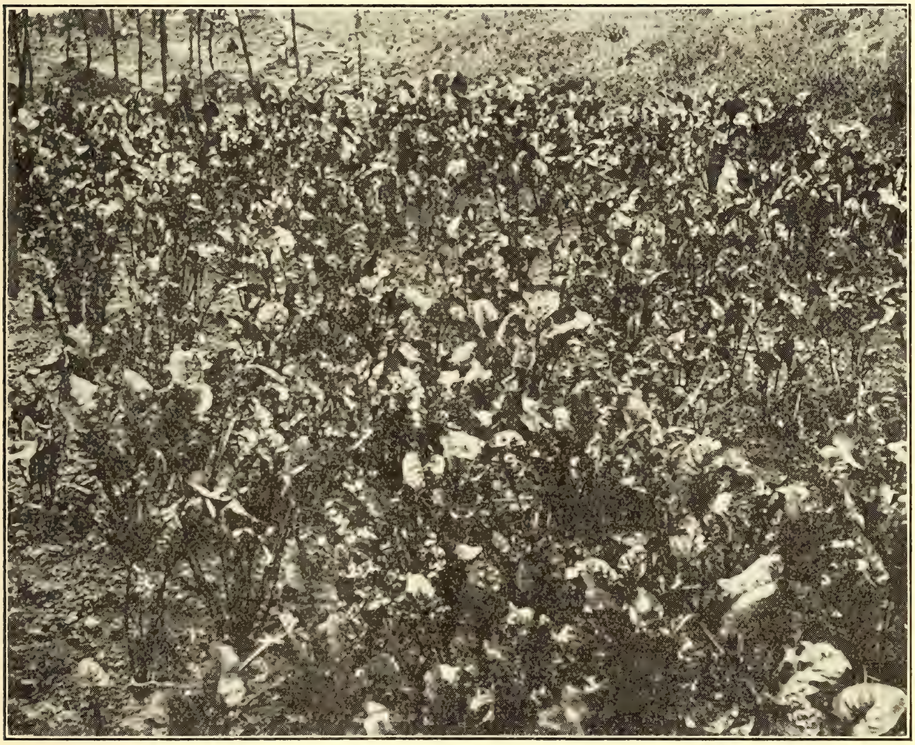


FIG. 3. A portion of plot "E" with the garden beet, one month after inoculating the root with *Bacterium tumefaciens*. The soil was well fertilized, and cultivated.

A difference in the size of the plants in the plots "E" and "SW" appeared very early. Figure 3 shows a portion of plot "E." The leaves are not only larger but more numerous. The rows appear to be indistinct owing to the fact that the leaves cover the ground. The plants in the plot at the time this picture was made had been inoculated one month. The difference between plots "E" and "SW" was even more pronounced at the time the crop was harvested.

Figure 4 shows part of the plot "SW" with the poor soil. The plants are relatively small and the leaves few. A considerable number of plants in this plot died. Here, also, the figure represents the condition of the plants in the plot one month after inoculation. An examination at this time of the roots of a few plants from plot "E," on good soil, showed that the inoculated plants had produced crown galls which were about the size of small hickory nuts, while those on the roots of the largest plants in poor soil, plot "SW," were barely in evidence or at most had attained a size equal to that of a pea.

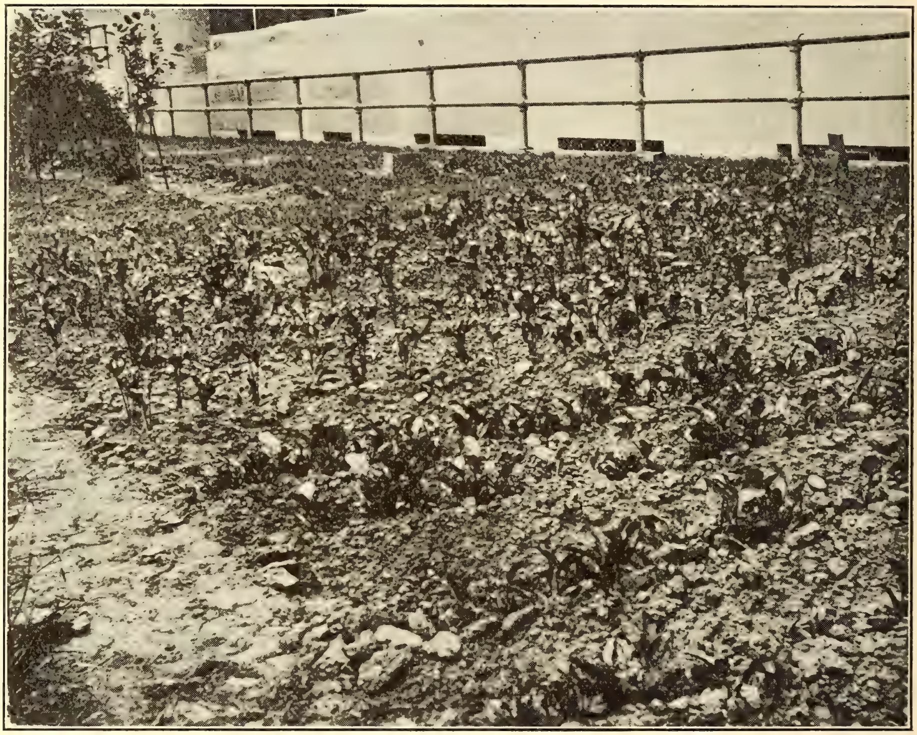


Fig. 4. A portion of plot "SW" with the garden beet, one month after inoculating the root with *Bacterium tumefaciens*; the soil was unfertilized and had never before been cultivated.

On examining the roots of the beets in October, when the entire group was harvested, the crown galls on the plants grown in the good soil, plot "E," were proportionately very large as compared to those on the roots of beets grown in plot "SW." Often the crown gall surrounded the entire upper portion of the root so that the normal contour of the root became indistinct. Figure 5 shows some typical roots with crown gall grown in good soil (plot "E").

The plants grown on poor soil (plot "SW") which were inoculated at about the same time as those growing in plot "E" were not only small, but the crown galls were proportionately small as compared to the size of the root. Figure 7 represents a series of beet roots grown in plot "SW," photographed at maturity when the plants were harvested; in this series the smallest crown gall was smaller than a pea while the largest was about the size of a black walnut.

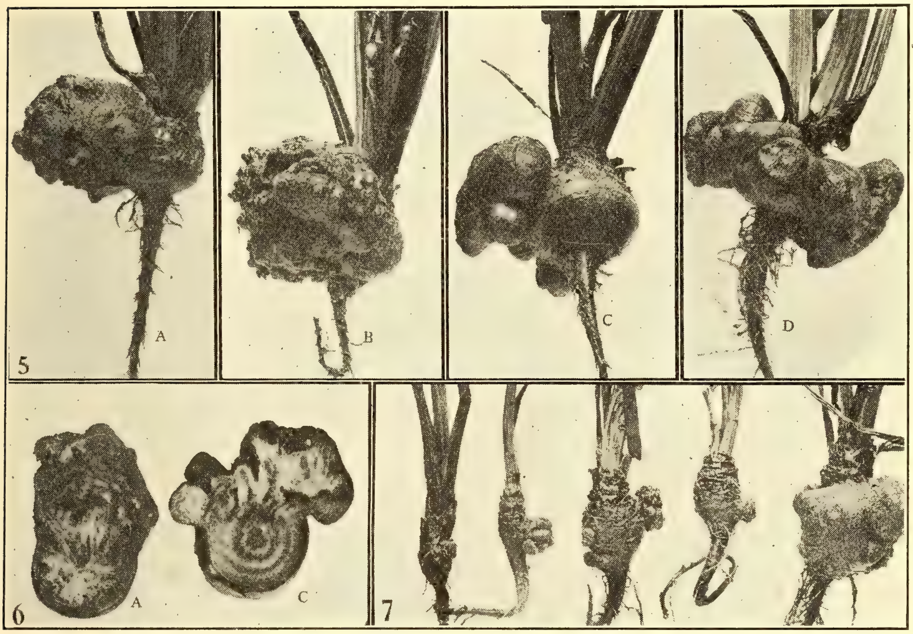


FIG. 5, A, B, C, D. A series of crown galls on the roots of the garden beet grown in fertilized soil; one month after inoculation. FIG. 6, A, C. Cross sections of the roots shown in figure 5, A and C. FIG. 7. A series of crown galls on garden beets grown in poor soil. Inoculation made August 11, 1920. Photographed October 16, 1920.

My observations on the appearance of the crown galls grown on these varieties of the garden beet are not in complete accord with those of Jensen, who claims that tumor-like growths on the different varieties of the garden beet differ from one another both in appearance and structure. In my cultures all the common types of crown gall appeared in each variety. Figure 5A represents the smooth type, 5B a warty gall, and 5C and 5D represent mixed types (smooth and warty), a form which Jensen does not recognize. In the mangel wurzel described below and shown in figure 9, we have similar types. The warty and the smooth types appear here as does the mixed type. It is of interest to note that crown galls of the smooth and warty types are also found in artificially produced crown galls on *Ficus elastica* (Levine, 1921.) The significance of these different types of crown gall is not yet perfectly understood.

In my observation of the galls on the Early Model and Egyptian Early varieties, a number of smooth galls were found to be stemless as shown in figures 6A and 6C, which are cross sections of the beets shown in figures 5A and 5C respectively. The warty galls are as a rule stemless. The galls in the plants grown in plot "SW" were also of the three types, and the arrangement of the vascular bundles in the crown galls appears to depend upon the internal structure of the root before inoculation.

Shortly after the plants of the two plots "E" and "SW" were gathered they were carefully washed and weighed. They were loosely wrapped in separate pieces of paper and dried in a hot air oven at 100° C. for several days and then weighed again. The results are given in table 2. Columns 1 and 2 give the fresh and dry weights of the normal plants from plot "SW," that is, in the unfertilized soil.

TABLE 2. *Comparison of weights of Beta vulgaris, vars. Early Model and Egyptian Early, grown on plots "SW" and "E," the roots of which were inoculated with Bacterium tumefaciens; controls were growing among the inoculated plants. The inoculations were made August 4, 1920; the crop was harvested October 19, 1920*

	Plants grown in Plot "SW" (unfertilized soil)				Plants grown in Plot "E" (fertilized soil)			
No.	Normal		Root Inoculated		Normal		Root Inoculated	
	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry
1	5.90	.50	24.30	1.90	22.20	1.55	11.50	1.10
2	3.70	.45	17.50	1.50	27.60	2.15	33.10	3.90
3	5.30	.65	2.50	.20	18.80	1.20	38.40	3.10
4	5.50	.65	8.40	.70	47.2	3.70	21.10	2.05
5	9.50	1.45	19.40	1.50	24.30	2.10	29.30	25.05
6	7.60	.80	9.80	.70	17.20	1.70	61.60	5.30
7	9.00	1.00	8.50	.60	28.30	2.70	17.10	1.20
8	23.80	2.30	16.80	1.50	27.10	2.60	56.10	4.60
9	29.80	3.80	5.00	.60	28.80	7.70	38.10	3.90
10	9.00	1.30	8.10	.60	19.60	4.20	38.00	3.80
11	24.00	3.30	10.90	.90	54.50	3.00	26.00	2.70
12	14.00	1.40	5.00	.50	37.50	2.50	24.10	1.50
13	5.40	.90	4.30	.40	42.20	.90	44.00	2.70
14	4.50	.50	7.20	.80	9.10	2.25	96.10	6.50
15	2.40	.30	7.90	.70	23.40	1.55	20.10	1.40
16	6.00	.60	5.20	.50	21.70	2.80	42.10	3.25
17	11.20	1.30	6.80	.65	34.60	1.00	33.50	2.40
18	39.00	3.50			13.00	5.40	32.50	4.50
19	9.00	1.90			79.20	3.50	83.10	7.00
20	10.00	.90			46.80	3.40	25.70	2.70
21	8.00	1.70			20.60	1.10	18.10	1.50
22	12.90	1.90			18.00	2.90	37.60	2.90
23	20.00	.45			33.50	6.10	71.50	6.00
24	20.50				69.50	.70	21.80	1.70
25	4.60				9.70		18.50	1.65
26							72.10	6.50
27							28.50	2.45
28							36.10	2.60
29							16.00	1.10
Totals	301.50	31.55	167.60	14.25	774.40	66.10	1,091.60	115.05
Averages	12.06	1.26	9.85	.83	30.96	2.64	37.64	3.96

The average weight of the entire plants when fresh is 12.06 g. The average dry weight is 1.26 g. The uninoculated plants grown in plot "E," that is, in fertilized soil, attained an average weight of 30.96 g. with a dry weight of 2.64 g. (columns 5 and 6). The difference in the weights of the normal plants can be interpreted only as due to differences in the amounts of available food in the soil.

The uninoculated plants in both plots were, as noted, scattered among those that had been inoculated. The average weight of the inoculated plants grown in unfertilized soil (plot "SW") is less than that of the healthy plants (see table 2, columns 3 and 4). This difference in favor of the normal plant indicates that the presence of the parasite in these cases has lowered the total growth (tissue-producing capacity) of the plant.

A slight variation in the number and size of the leaves affects the total weights of the plants, yet the beet roots of the same variety bearing crown galls are always larger and weigh more than the normal roots grown under similar conditions. The marked difference in weight between the inoculated plants grown in plot "E" (fertilized soil, see table 2, columns 7 and 8) and the inoculated plants grown in plot "SW" is of considerable interest and not only indicates a difference in the nutritive conditions of the plants but confirms the data already obtained for these plants when grown in pots, and also supports the contention that the healthy plant responds to the influence of an invading organism more vigorously than does the poorly nourished or less robust plant.

These results further support the view maintained by many plant and animal pathologists that the response to an invading organism is greatest and that the parasite is most favored when the necessary metabolic processes of the host are satisfied.

THE WEIGHT OF THE CROWN GALL COMPARED WITH THE WEIGHT OF THE PLANT

I have further studied the weight of the crown-gall tumors from both the large and the small beets. The varieties used were Early Model, Egyptian Early, and Giant Mangel Wurzel. The plants in this experiment were grown under the most favorable conditions. When the tap roots had developed they were inoculated with *Bacterium tumefaciens* on August 4, 5, and 6, 1920, in the manner described above and were harvested October 15, 1920. An equally large number of plants among the inoculated ones were left to serve as controls. Figure 8 represents a portion of plot "W" in which the Giant Mangel Wurzel was growing.

For convenience, the crown gall was considered as that part of the root which could be separated from it by a stroke of the knife, continuous with the normal contour of the root. It is readily seen that this does not remove all the crown gall, for the neoplasia in the plant also invades the normal tissues, as Levin and Levine (1920) have shown for other plants, so that the weights of the crown galls in tables 3 and 4 are somewhat below the actual weights; while the weights of the roots given in these tables are above the actual weights of the normal root tissue.

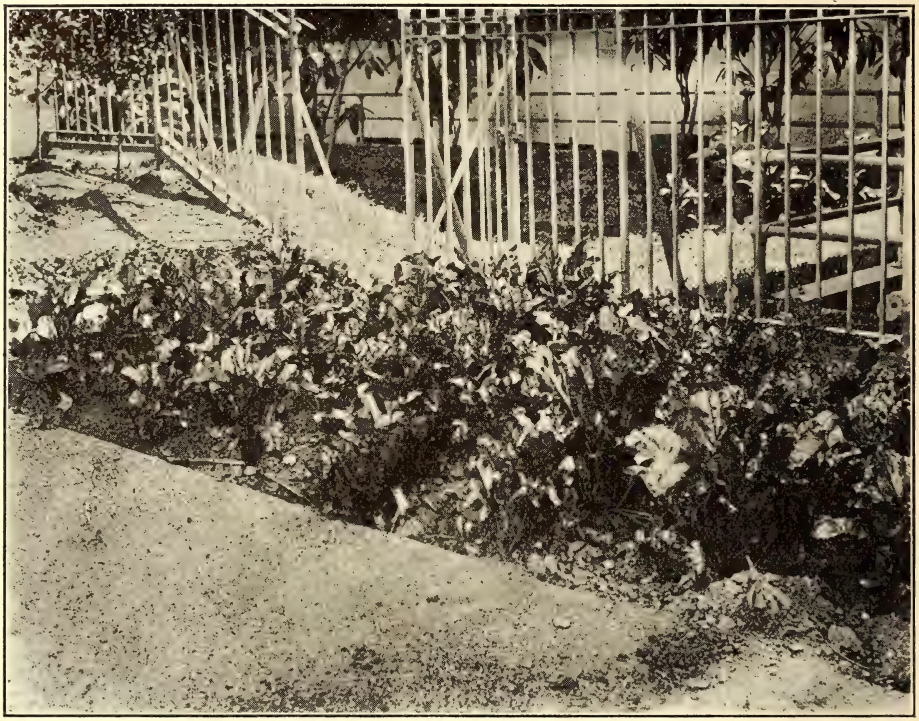


FIG. 8. A portion of plot "SW" showing the mangel wurzel, one month after the roots were inoculated with *Bacterium tumefaciens*.

The weight of the crown gall was determined by subtracting the weight of the plant, with the crown gall removed, from the weight of the entire plant. The weight of the leaves was determined by subtracting the weight of the root from that of the plant minus the crown gall.

The normal or uninoculated garden beet, varieties Early Model and Egyptian Early, showed an average weight of 39.49 g. (see table 3). The weight of the average normal root is 11.98 g., while that of the crown is 27.51 g. The entire weight of the average inoculated plant was 32.94 g., which is slightly below the normal weight. The average weight of these plants with the crown galls removed is 23.69 g., and the average weight of the crown galls is 9.25 g. The root without the crown gall averaged 8.61 g., the average weight of the crown gall and root is 17.86 g., which is above the average weight of the normal or uninoculated root. The decrease in size of the crown in the inoculated plants explains the difference in weight between the normal and the inoculated plants. I have not undertaken at this time to explain the cause for the difference in these crowns. It may be suggested that possibly the disturbance of the vascular tissues of the root by the crown gall may be responsible for it, but no definite evidence is at hand.

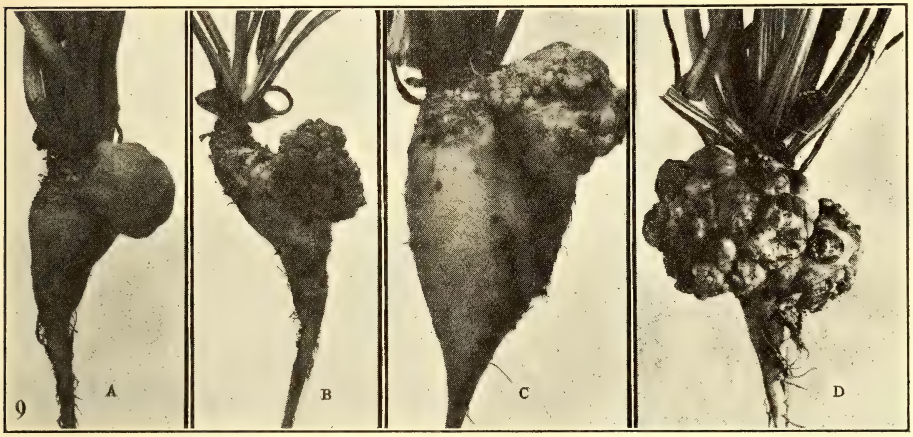


FIG. 9, A, B, C, D. Types of crown gall on the mangel wurzel: A, smooth; B, warty; C, smooth-warty condition combined; D, small smooth galls covered with clusters of warts.

The weights of the inoculated and uninoculated mangel wurzel show similar conditions. The average weight of the uninoculated mangel wurzel grown in these cultures is 113.47 g. The weight of the root is 52.09 g., and the average weight of the crown is 61.38 g.

The average weight of the inoculated plants, samples of which are shown in figure 9 with the smooth, warty, and mixed galls, is 124.38 g. (see table 4), which is far above the average weight of the normal plant. The weight of the plant without the mass of the crown-gall tissue is 84.31 g., while that part of the crown gall that could be removed from the root averaged 39.91 g. The average weight of the root is 41.69 g. and of the crown 42.78 g. The average weight of the root and its crown gall is 81.6 g. Here again the crown gall increased the weight of the root as in the other cases mentioned above; while the weight of the leaves in the inoculated plants was considerably lower, the growth of the root overcame the difference so that the average weight of the entire inoculated plants was greater than that of the normal plants.

It may be of further interest to note that the crown galls in the mangel wurzel, harvested approximately two months after inoculation, weighed more than the remainder of the root. None of the crown gall tissue on the mangel wurzel had disintegrated at the time they were harvested, but on being brought into the laboratory and moistened, decay set in at once. The crown galls alone turned black, and within 48 hours they were disintegrating while the normal tissues of the root remained unchanged.

TABLE 3. *Weights of the leaf, root, and crown gall of Beta vulgaris, varieties Early Model and Egyptian Early, compared with the weight of the entire plant when grown under favorable conditions*

No.	Normal			Root Inoculated August 15, 1920		Harvested October 20, 1920		
	Total	Root	Leaf	Total	Without Gall	Gall	Root	Leaf
1	32.50	8.30		18.60	13.80		5.00	
2	137.70	33.80		52.50	30.60		6.80	
3	32.20	5.10		56.20	52.50		18.40	
4	10.00	3.00		19.00	11.70		5.60	
5	20.70	7.00		48.00	22.70		13.20	
6	19.10	2.50		108.80	100.90		18.00	
7	13.90	3.70		75.30	59.70		20.20	
8	22.20	2.20		57.40	47.80		23.40	
9	20.50	4.60		20.20	7.30		4.20	
10	16.70	3.00		13.90	13.00		2.10	
11	24.00	5.00		20.00	13.50		6.70	
12	13.50	4.20		30.00	19.50		6.00	
13	14.20	2.30		98.75	75.50		18.90	
14	160.00	29.70		27.50	15.30		6.10	
15	49.70	14.30		9.40	5.60		2.20	
16	71.70	13.90		23.60	15.60		5.10	
17	36.80	4.10		29.60	16.10		8.40	
18	15.90	2.90		93.00	64.30		35.50	
19	69.00	85.00		122.00	105.00		57.40	
20	9.50	5.00		72.20	54.20		17.85	
21				26.70	17.60		4.10	
22				17.80	9.00		2.90	
23				20.80	17.60		3.80	
24				37.10	30.70		6.90	
25				33.50	28.20		6.90	
26				18.40	11.80		14.40	
27				29.60	10.85		4.20	
28				17.60	9.60		6.50	
29				32.60	18.50		3.80	
30				42.70	37.30		5.70	
31				11.80	9.90		6.70	
32				23.00	20.30		2.90	
33				14.00	7.80		2.90	
34				19.30	15.90		1.60	
35				15.10	6.70		3.00	
36				59.00	47.60		3.70	
37				40.10	25.10		16.20	
38				38.40	16.30		18.00	
39				42.50	23.30		4.50	
40				31.30	22.50		6.70	
41				9.60	8.60		6.50	
42				11.70	11.00		4.70	
43				135.50	121.00		4.50	
44				20.90	18.20		19.90	
45				35.60	31.00		10.10	
46				38.00	29.00		16.00	
47				18.10	0.00		8.90	
48				45.60	24.30		3.50	
49				13.65	19.70		14.70	
50				46.60	34.10		15.40	
51				656.85	11.30		8.80	
52				21.50	24.90		5.95	
53				36.00	477.80		10.70	
54				13.70	9.60		175.05	

No.	Normal			Root Inoculated August 15, 1920		Harvested October 20, 1920		
	Total	Root	Leaf	Total	Without Gall	Gall	Root	Leaf
55				18.80	23.70		3.50	
56				23.50	8.00		5.80	
57				13.80	14.60		4.50	
58				12.80	12.00		7.80	
59				13.40	9.00		3.90	
60				22.05	8.90		4.00	
61				11.80	8.10		1.80	
62				37.50	17.35		3.30	
63				25.35	8.80		8.50	
64				12.90	27.40		3.40	
65				15.30	15.80		20.50	
66				15.30	9.50		2.70	
67				11.70	9.30		1.80	
68				17.70	11.10		3.40	
69				20.10	8.30		2.40	
70				15.50	9.50		4.00	
71				15.80	19.00		4.30	
72				18.00	8.60		4.20	
73				392.50	7.30		3.70	
74					9.70		1.45	
75					255.55		4.70	
76							99.65	
Total	789.80	239.60	550.20	2,405.30	1,729.70	675.60	628.65	1,101.05
Averages	39.49	11.98	27.51	32.94	23.69	9.25	8.61	15.08

Discussion of Results

The average weight of the root of the normal beet grown in fairly fertile soil is less than the average weight of the root of the same variety bearing a crown gall due to an artificial inoculation with *Bacterium tumefaciens* and grown under similar conditions. The average weight of the beets bearing crown galls and grown in pots filled with a garden soil and manure combination is greater than the average weight of the beets inoculated with *Bacterium tumefaciens* and grown in pots filled with sand. The average weight of the entire normal beet plants is greater or less than the average weight of the entire inoculated beet plant, depending upon the number of leaves on each. The leaves and crown of a normal beet plant weigh more than the leaves and crown of a plant of the same variety grown under similar conditions but whose root bears a crown gall. It is suggested that this difference in leaf development may be caused by interference with the distribution of food caused by the crown galls, although no direct evidence upon this question is available.

The evidence is clear that a well nourished, vigorously growing, and healthy host responds to the invasion of a parasite by a hypertrophy and a hyperplasia which are greater than result in the case of a poorly nourished or feebly growing host. These results are in agreement with the observations of other workers as to the relations of host and parasite in certain cases.

TABLE 4. *Relation of the weights of the leaf, root, and crown gall of the Giant Mangel Wurzel to the weight of the entire plant when grown under favorable conditions*

	Normal			Roots Inoculated August 5, Harvested October 9, 1920				
No.	Total	Root	Leaf	Total	Without Gall	Gall	Root	Leaf
1	67.10	32.00	35.10	143.90	63.30		34.60	
2	123.40	68.10	55.30	50.30	33.70		28.70	
3	117.40	41.40	76.00	51.10	39.90		18.80	
4	102.80	37.40	65.40	45.70	21.30		14.20	
5	28.50	4.10	24.40	199.60	129.60		83.30	
6	76.60	14.30	62.30	250.80	170.30		80.20	
7	131.00	58.70	72.30	210.20	140.10		92.00	
8	81.05	55.60	25.45	135.90	96.80		42.90	
9	230.60	101.90	128.70	193.60	118.50		63.60	
10	114.40	38.50	75.90	51.10	36.40		21.10	
11	121.00	56.90	64.10	176.10	133.00		60.00	
12	226.30	144.50	81.80	163.70	76.60		37.80	
13	174.50	95.75	78.75	42.30	24.70		17.80	
14	75.00	35.50	39.50	111.80	60.80		22.20	
15	77.10	36.20	40.90	78.60	58.20		41.80	
16	82.40	32.30	50.10	85.60	61.05		39.10	
17	100.00	32.50	69.60	61.40	36.20		23.30	
18				58.90	40.80		26.00	
19				50.60	38.90		19.55	
20				86.40	38.70		22.70	
21				187.70	146.05		88.05	
22				86.70	78.40		32.70	
23				30.70	24.50		14.15	
24				38.20	37.70		16.10	
25				125.80	115.70		40.00	
26				190.00	116.35		73.55	
27				410.00	264.60		106.40	
28				31.05	25.80		15.20	
29				111.40	61.00		36.60	
30				152.90	109.00		43.50	
31				164.00	115.10		62.10	
32				121.30	89.20		51.00	
33				199.40	115.90		67.90	
34				83.35	57.10		28.00	
35				25.00	22.50		14.40	
36				110.60	73.00		44.30	
37				120.50	79.70		28.00	
38				32.40	24.00		13.00	
39				75.40	39.50		27.80	
40				209.40	142.30		47.00	
41				104.60	65.20		26.80	
42				57.40	34.70		27.00	
43				28.80	30.50		13.60	
44				178.00	84.00		65.50	
45				74.40	53.70		20.30	
46				25.60	13.20		2.80	
47				243.40	173.40		10.50	
48				211.50	186.00		122.00	
49				276.50	258.10		83.10	
50				154.50	135.30		43.85	
51				161.00	105.00		55.00	
52				200.90	137.70		97.05	

	Normal			Roots Inoculated August 5, Harvested October 9, 1920				
No.	Total	Root	Leaf	Total	Without Gall	Gall	Root	Leaf
53				51.50	46.40		14.00	
54				118.40	64.20		38.70	
55				68.70	40.50		24.00	
56				137.60	103.10		42.10	
57				129.00	73.80		43.60	
58				198.60	123.40		62.90	
59				165.50	90.00		28.10	
Totals	1,929.15	885.65	1,043.50	7,339.30	4,984.45	2,354.85	2,459.90	2,524.55
Averages	113.47	52.09	61.39	124.38	84.31	39.91	41.69	42.78

Spinks (1913) in his study of water and soil cultures of wheat and barley showed that these plants were more susceptible to *Puccinia glumarum* and *Erysiphe graminis* when the plants were provided with large amounts of available nitrogen. Plants which were semi-starved as regards nitrogen exhibited a considerable degree of so-called immunity. Peltier (1918) and Peltier and Frederich (1920) showed that in the case of citrus canker the hosts were more susceptible when placed in conditions that induced rapid and vigorous growth.

Fromme and Murray (1919), studying the angular leaf spot of tobacco, a bacterial disease, found that those factors which promote rapid and vigorous growth of the host favor the parasite, and again Thomas (1921), studying the relation of the health of the host *Apium graveolens* (celery) to infection with the fungus *Septoria apii*, observed that those conditions of temperature, feeding, etc., which favored the health of the host as evidenced by vigorous development also increased the number of infections on the leaves.

Crown gall is analogous to animal cancer, as maintained by Smith, Levin and Levine. In the case of animal cancer, Ewing holds that "good health appears to favor the growth of tumor grafts and poor conditions retard it," while Teague claims that he was able to transplant mammary carcinoma of dogs only in animals weakened with distemper. In this relation of host vigor to virulence of the disease, crown gall, potato wart, club root, and other plant diseases resulting in hypertrophy and hyperplasia which have not been studied from this point of view are analogous to animal cancer.

This does not argue for or against the parasitic origin of animal cancer, for while the end results in animal and plant cancers are analogous, the initial stimuli may be entirely different—parasitic, mechanical, or even chemical.

The correlative of this proposition is quite true also, for Miss Brown (1920) has showed that *Pestalozzia* sp. inoculated into Sapodilla may produce a large swelling, apparently a crown gall, while in larch, hemlock, and blue spruce this organism produces blighting of the leaves and no galls.

Crown gall belongs in the great class of diseases involving hypertrophies and hyperplasias of the host tissue. The reaction of the host to the parasite in these cases is obvious and specific. In sharp contrast with these we have the great group of cases in which plants parasitized by fungous or bacterial parasites show susceptibility by lack of visible reaction. Their tissues become necrotic because of destruction by the invading parasite. Whether in the cases of crown gall, potato wart, club root, and many other diseases the reaction appearing in the form of neoplastic growths may be interpreted as actively protective, is a difficult question. The types of disease in the two cases are certainly sharply distinct in plants; the symptoms in the former indicating accelerated metabolic activity and growth, while in the second case we have necrosis such as the soft rots, dry rots, cankers, etc.

We need not accept the conception advanced by some plant pathologists that the better the health of the organism the greater the susceptibility to an invading parasite. Susceptibility implies a certain relative adaptation to serve as a substratum for the growth of the parasite. The data presented as to crown galls on beets do not bear on the question of susceptibility. Under the conditions described the beets all showed practically 100 percent susceptibility. It is, however, in my opinion clearly shown from the results described above that the invading parasite induces a visible reaction in direct proportion to the general health of the individual. The proliferation of cells in the region of the invasion depends upon the general vigor and capacity for growth of the host tissue. This is in marked contrast with those plant diseases in which disintegration of the tissues is the first obvious evidence of the presence of the parasitic germ.

SUMMARY

1. *Beta vulgaris*, varieties Early Model, Egyptian Early, and Giant Mangel Wurzel, were grown in different kinds of soil to test the effect of the soils on the growth, size, and weight of the root when artificially inoculated with *Bacterium tumefaciens*.

2. Of pot cultures with (1) garden soil with an abundance of manure, (2) brown silt loam and manure, (3) brown silt loam, and (4) medium sand, the largest average weight of plants was obtained in the garden soil. The crown galls were also the largest on these plants. The plants grown in sand weighed the least, were the smallest in size, and had the smallest crown galls.

3. Beets grown in open-air plots gave the same results. Those on the better soil were larger and heavier, and the crown galls on these roots were larger than those on beets grown in the poorer soil.

4. While the weight of the individual plants both inoculated and unin-

oculated varied widely, the average weight of the roots with crown gall was greater than the average weight of the normal or uninoculated roots in the same plot.

5. Three different types of crown gall were observed in *Beta vulgaris*, namely, the smooth type, the warty type, and a mixed type consisting of the warty and smooth types.

6. The reaction of the beet to crown gall depends upon the health of the beet; that is, with any given lot of seeds the extent of the reaction and the size of the crown gall depend ultimately upon the condition of the soil and upon other environmental factors.

MONTEFIORE HOSPITAL,
NEW YORK CITY

LITERATURE CITED

Brown, N. A. 1920. A Pestalozzia producing a tumor on the Sapodilla tree. Phytopath. **10**: 383–394. figs. 1–5.

Ewing, J. 1919. Neoplastic diseases. Philadelphia.

Fromme, F. D., and Murray, T. J. 1919. Angular leaf spot of tobacco, an undescribed bacterial disease. Jour. Agr. Res. **16**: 219–228. Pls. 25–27.

Jensen, C. O. 1918. Undersøgelser vedrørende nogle Svulstlignende Dannelser hos Planter. Meddelelser Fra Den Kgl. Veterinaer-Og. Landbohøjskoles Serum Laboratorium **54**: 91–143. figs. 1–17. Pl. 1.

Kunkel, L. O. 1918. Tissue invasion by *Plasmodiophora brassicae*. Jour. Agr. Res. **14**: 543–572. Pls. 61–80.

Levin, I., and Levine, M. 1920. Malignancy of the crown gall and its analogy to animal cancer. Jour. Cancer Res. **5**: 243–260. figs. 1–15.

Levine, M. 1919. Studies on plant cancers I. The mechanism of the formation of the leafy crown gall. Bull. Torrey Bot. Club **46**: 447–452. Pls. 17, 18.

——. 1920. The behavior of crown gall on the rubber tree (*Ficus elastica*). Proc. Soç. Exp. Biol. Med. **17**: 157–158.

——. 1921. Studies on plant cancers II. The behavior of crown gall on the rubber plant (*Ficus elastica*). Mycologia **13**: 1–11. Pls. 1, 2.

Magnus, W. 1918. Wund Callus und Bacterien Tumore. Ber. Deutsch. Bot. Ges. **36**: 20–29.

Peltier, G. L. 1918. Susceptibility and resistance to citrus-canker of the wild relatives, citrus fruits, and hybrids of the genus Citrus. Jour. Agr. Res. **14**: 337–357. Pls. 51–53.

Peltier, G. L., and Frederich, W. J. 1920. Relative susceptibility to citrus-canker of different species and hybrids of the genus Citrus, including the wild relatives. Jour. Agr. Res. **19**: 339–362. Pls. 57–68.

Orton, C. R., and Kern, F. D. 1919. The potato wart disease. Pa. State Coll. Agr. Exp. Sta. Bull. **156**: 1–16. figs. 1–4.

Smith, E. F., Brown, N. A., and Townsend, C. O. 1911. Crown gall of plants, its cause and remedy. U. S. Dept. Agr. Bur. Plant Ind. Bull. **213**: 1–215. Pls. 1–35.

Smith, E. F., Brown, N. A., and McCulloch, L. 1912. The structure and development of crown gall. U. S. Dept. Agr. Bur. Plant Ind. Bull. **255**: 1–60. Pls. 1–109.

Spinks, G. T. 1913. Factors affecting susceptibility to disease in plants. Jour. Agr. Sci. **5**: 231–247. Pl. 7.

Thomas, H. E. 1921. The relation of the health of the host and other factors to infections of *Apium graveolens* by *Septoria apii* Rostr. Bull. Torrey Bot. Club **48**: 1–29.

Townsend, C. O. 1915. Field studies of the crown gall of the sugar beets. U. S. Dept. Agr. Bur. Plant Ind. Bull. **203**: 1–8. Pls. 1, 2.

INDEX TO VOLUME VIII

(New names and final members of new combinations are in **heavy face** type.)

Abutilon berlandieri, 195; *incanum*, 195; *jacquini*, 195; *malacum*, 199; *parvulum*, 199; *pedunculare*, 195; *texense*, 198; *triquetrum*, 195; *wrightii*, 195
Acacia, 197
Aceraceae, 330
Achlya, 231; *paradoxa*, 236
Actaea, pollen tubes of, 474
Aecidium Dicentrae, 445
Agave, 198, 218
Agrostemma, pollen tubes of, 472, 477
Aira, 255; *caespitosa*, 255; *caryophyllea*, 255
Alisma, embryogeny of, 505; pollen of, 472
Alnus, pollen of, 472; *glutinosa*, pollen of, 479
Alternaria, 320
Althaea rosea, 201
Alveolaria, 447
Amaryllidaceae, 331, 387
Anacardiaceae, 330
Anagallis, stylar canal of, 475
Andropogon, 193; *alopecuroides*, 48; *divaricatum*, 48; *hirtus*, 255; *virginicus*, 255
Anemone, pollen of, 472
Anions, effect upon permeability of polyvalent cations in combination with polyvalent, 382; quantitative study of the effect of, on the permeability of plant cells, 366
Anonaceae, 330
Antiaris toxicaris, 238
Antirrhinum, 472; *tortuosum*, pollen of, 479
Apium graveolens, infected with *Septoria apii*, 523
Aplopsora, description of genus, 442; *Nyssae*, 442
Apocynaceae, 330
Apple, pollen of, 480
Araceae, 330; food plants of, 329
Araucaria brasiliana, 219; *imbricata*, 226
Araucarioxylon Hoppertoniae, 224
Aristolochia Sipho, 185
Arthur, Joseph Charles. Specialization and fundamentals in botany, 275
Arundinaria Simoni, 219
Asclepiadaceae, 330
Aspergillus aurantiacus, 122; *aureus*, 123; *chrysospermum*, 119; *citrisporus*, 119; *effusus*, 105; *flavescens*, 112; *flavus* and associated species, 103; *flavus-oryzae*, 103; *fulvus*, 120; *fumigatus*, 109; *giganto-sulphureus*, 107; *glaucus*, 111; *gymnosardae*, 108; *luchuensis*, 117; *micro-virido-citrinus*, 107; *nidulans*, 123; *niger*, 110; effect of salt proportions and concentration on the growth of, 147; *oryzae* and associated species, 103; *oryzae* var. *basidiferens*, 108; *parasiticus*, 105; *perniciosus*, 107; *pseudoflavus*, 108; *repens*, 111; *Siebenmanni*, 112; *tamari*, 103; *terricola*, 114; var. *Americana*, 114; *Tokelau*, 122; *umbrinus*, 120; *variabilis*, 126; *Wehmeri*, 111; *Wentii*, 103
Atropa belladonna, stylar canal of, 475
Avena sativa aristata, 259; *sterilis*, 456
Azotobacter, 1, 24; *beyerinckii*, 25; *chroococcum*, 24; *vinlandii*, 25

Bacillus fluorescens liquefaciens, 478; *radicicola*, 26
Bacteria, soil, 1
Bacterium tumefaciens, cancer of beet produced by, 507; *beticolum*, 508
Baeodromus, 447
Bald cypress, 198
Ball, Carleton R. The relation of crop-plant botany to human welfare, 323
Bambusa arundinacea, 219; *tesselata*, 219
Banana wilt, losses from, 315
Baptisia, 193
Bastardia viscosa, 195
Bechtel, Albert Reiff. The floral anatomy of the Urticales, 386
Beet, 33, 37, 257
Berberis Thunbergii, leaves of, 433; *vulgaris*, 433
Bergman, H. F. The effect of cloudiness on the oxygen content of water and its significance in cranberry culture, 50
Beta vulgaris, the nature of the soil as a determining factor in the health of the beet, and its relation to the size and weight of the crown gall produced by inoculation with *Bacterium tumefaciens*, 507; pollen of, 480
Betulaceae, 331

Bicuculla Cucullaria, 445
Boehmeria cylindrica, 398
Bollworm, pink, 192
Botrychium, 304; *obliquum*, 307; *Virginianum*, 306
BRANNON, J. M. A simple method for growing plants, 176
Brodiaea, 144
Bromeliaceae, 330
Bromus ciliatus, 48; *purgans*, 48
Brownea, pollen of, 472
Brown-rot of peaches, losses from, 316
Brucea furruginea, tyloses in, 184
Bryophyllum calycinum, 291
Bubakia, 447
Buckwheat, 328

Cacti, 198
Caeoma strobilina, 444
Caesalpinioideae, 175
Callirrhoe digitata, 195; *involucrata*, 194; *lineariloba*, 197
CAMPBELL, D. H. The eusporangiate ferns and the stelar theory, 303
Cancers, studies on plant, III. The nature of the soil as a determining factor in the health of the beet, *Beta vulgaris*, and its relation to the size and weight of the crown gall produced by inoculation with *Bacterium tumefaciens*, 507
Cannabinaceae, 331
Cannabis sativa, 395
Carduus, genus, 283
Carnation rust, 316
Cassia, 175
Castanea, Aspergillus cultures on bark of, 113; *dentata*, leaves of, 433
Cations, effect upon permeability of polyvalent, in combination with polyvalent anions, 382
Cedar, mountain, 198
Celtis occidentalis, 393; *pallida*, 194
Cenchrus spicatus, 47
Centaurea orientalis, 31
Cercis canadensis, 173, 174, 175
Cercospora, 320
Cereus grandiflorus, pollen tubes of, 474
Cerotelium Canavaliae, 449; **Dicentrae**, 445; *Fici*, 450; *Gossypii*, 450; *Vitis*, 450
Chaetochloa italica, 43; *lutescens*, 41; *viridis*, 48
CHASE, AGNES. The Linnaean concept of pearl millet, 41
Chenopodiaceae, 328, 330
Chestnut-bark disease, loss from, 316
CHILD, C. M. Certain aspects of the problem of physiological correlation, 286
Chlorella sp., 2, 14, 15; *vulgaris*, 2, 14, 15
Chlorophyceae, 1, 2, 27; cultures of, 2
Chnoopsora, 443
Chrysanthemum rust, 316
Chrysomyxa, 447
CHURCH, MARGARET B. (See Charles Thom, 103)
Chusquea abietifolia, 219
Cienfuegosia sulphurea, 195
Cirsium, 283; *arvense*, 283
Cistus, stylar canal of, 475
Citrus, 219; canker, 316, 319; *grandis*, 433
Clarkia, 144
Climbing bamboo, 219
Clivia, pollen of, 472
Clostridium, 1
Cloudiness, the effect of, on the oxygen content of water and its significance in cranberry culture, 50
Cnicus, genus, 283
Cocoanut palm bud-rot, losses from, 315
Coffee rust, losses from, 315
Colchicum autumnale, pollen tubes of, 474
Collinsia, 144
Colocasia, 370
Compositae, 330; food plants of, 329
Condalia obovata, 197
Conifers, 314
Convolvulus, pollen of, 472
Corn, pollen of, 477, 483
Correlation between bundle number in siblings, 357
Correlation, physiological, certain aspects of the problem of, 286
Correlations between anatomical characters in the seedling of *Phaseolus vulgaris*, 339
Corypha, 219; *umbraculifera*, 219
Cotinus coggygria, 184
Cottonwood, 199
Covillea tridentata, 197
Cranberry culture, the effect of cloudiness on the oxygen content of water and its significance in, 50
Crocus, pollen of, 472; pollen tubes of, 474
Crop-plant botany, relation of, to human welfare, 323
Crown gall of beet, 507
Crown rust of oats, miscellaneous studies on the, 452

Cruciferae, 330; food plants of, 329
Cucurbit mildew, 316
Cucurbitaceae, food plants of, 329; stylar canal of, 475
Cyanophyceae, 1
Cyathus Poeppigii, 282
Cycadella wyomingensis, 225
Cycadeoidea colossalis, 225; *dacotensis*, 224; *Dartoni*, 222; *Fisherae*, 221; *ingens*, 222; *marshiana*, 225; *marylandica*, 221; *Masseiana*, 221; *nigra*, 221; *Reichenbachiana*, 224; *Stantoni*, 221; *Stilwelli*, 225; *Uddeni*, 227
Cycadeoids, monocarpy and pseudomonocarpy in the, 218
Cyclamen, 33, 34
Cypress, pollen of, 479
Cypripedium, 248
Cyrtanthus parviflorus, embryogeny of, 502; *sanguineus*, 502
Cystococcus, 25, 26

Daisy, pollen of, 483
Danaea, 308; *elliptica*, 310; *Jamaicensis*, 310; *simplicifolia*, 304
Dandelion, pollen of, 483
Dasylirion, 199
Date palm, pollen of, 474
Dickson, James Geere. The relation of certain nutritive elements to the composition of the oat plant, 256
Dietelia, 449
Disella hederacea, 200; *lepidota*, 198; *sagittaefolia*, 200
Dock, pollen of, 477
Dunn, Grace A. Note on the histology of grain roots, 207
Durham, G. B. (See J. Arthur Harris, 63, 339, 375, 425)

Ebenaceae, 330
Ehretia elliptica, 194
Elm, pollen tubes of, 474
Elodea, respiration and antagonism in, 458; *canadensis*, 458
Embryogeny of *Cyrtanthus parviflorus* Baker, 502
Emerson, R. A. Genetic evidence of aberrant chromosome behavior in maize endosperm, 411
Enckea, 212
Ensign, M. R. Area of vein-islets in leaves of certain plants as an age determinant, 433

Enzymes, pollen, 471
Ephedra trifurca, fertilization of, 476
Equisetum, 289
Eragrostis, 193
Ergots, 319
Erianthus, 48; *divaricatus*, 48
Ericaceae, 331
Eryngium campestre, 31
Erysiphe graminis on wheat and barley, 523
Eucharis, pollen of, 472
Eurotium, 123; *oryzae*, 124; *repens*, 125
Eusporangiate ferns and the stelar theory, 303

Fagaceae, 330
Fagus caroliniana, leaves of, 433
Ficus elastica, crown gall on, 515
Filicineae, 303
Fixation of free nitrogen by green plants, the, 1
Flag smut of wheat, 316, 319
Floral anatomy of the Urticales, 386
Fouquiera splendens, 199
Fraxinaceae, 331
Fucus, 289
Furcraea americana, 219

Gayoides crispum, 197
Genetic evidence of aberrant chromosome behavior in maize endosperm, 411
Geraniales, 405
Gericke, W. F. Influence of temperature on the relations between nutrient salt proportions and the early growth of wheat, 59
Ginsburg, S. (See L. Knudson, 164)
Gladiolus, pollen of, 472
Gleditsia and related genera, thick-walled root hairs of, 171
Gleditsia triacanthos, 171, 173; var. *inermis*, 174
Gleichenia, 312, 313
Godetia, 144
Goldenrod pollen, 477
Grains, losses from disease, 315
Gramineae 328
Grape anthracnose, 316; black-rot, 316; downy mildew, 316
Growing plants, a simple method for, 176
Growth of *Aspergillus niger*, effect of salt proportions and concentration on the, 147
Gymnocladus dioica, 173, 174, 175
Gymnosporangium, 319

Hackberries, 198
Haenseler, C. M. The effect of salt proportions and concentration on the growth of *Aspergillus niger*, 147
Halophila, pollen of, 473
Hanson, Herbert C. Distribution of the Malvaceae in southern and western Texas, 192
Harris, J. Arthur, Edmund W. Sinnott, John Y. Pennypacker, and G. B. Durham. The vascular anatomy of dimerous and trimerous seedlings of *Phaseolus vulgaris*, 63; correlations between anatomical characters in the seedling of *Phaseolus vulgaris*, 339; the vascular anatomy of hemitrimerous seedlings of *Phaseolus vulgaris*, 375; the interrelationship of the number of the two types of vascular bundles in the transition zone of the axis of *Phaseolus vulgaris*, 425
Hazel, pollen tubes of, 474
Hedera trifolia Canadensis, 127
Helleborus, pollen of, 472
Hemitrimerous seedlings of *Phaseolus vulgaris*, vascular anatomy of, 375
Hemp, Mauritius, 219
Hibiscus cardiophyllus, 195; *coccineus*, 202; *coulteri*, 197; *denudatus* var. *involucellatus*, 199; *esculentus*, 202; *lasiocarpus*, 194; *manihot*, 202; *militaris*, 194; *rosa-sinensis*, 202; *sabdariffa*, 202; *syriacus*, 202; *trionum*, 202
Hickory, pollen tubes of, 474
Hicoria, 194
Hitchcock, A. S. The type concept in systematic botany, 251
Hoerner, G. R. Miscellaneous studies on the crown rust of oats, 452
Holcus lanatus, 253; *spicatus*, 44
Hordeum, 255; *vulgare*, 255
Horse chestnut, pollen of, 480
Huisache, 194
Humulus Lupulus, 396
Hyacinth, pollen of, 471
Hydration, water deficit and the action of vitamines, amino-compounds, and salts on, 296
Hymenophyllaceae, 313
Hypericum, stylar canal of, 475

Ilex, 194
Indian corn, roots of, 210
Ipomoea, pollen of, 472
Ipo toxicaria, 238
Iresine, 33, 37, 166, 168; *Herbstii*, 164
Iris, stylar canal of, 475; *pumila*, 31; *versicolor*, pollen tubes of, 474
Isoachlya, a new genus of the Saprolegniaceae, 231; *monilifera*, 231; *paradoxa*, 231; *toruloides*, 231

Johnson grass, 44
Juglandaceae, 330
Juglans regia, ovule of, 403
Juniperus, 199; pollen of, 479

Kauffman, C. H. Isoachlya, a new genus of the Saprolegniaceae, 231
Knudson, L., and S. Ginsburg. Suggestions with respect to the measurement of osmotic pressure, 164
Kosteletzkya althaeifolia, 194
Kuehneola, 450; *aliena*, 450; *Butleri*, 450

Labiatae, 330
Laminaria, 367, 382; *Agardhii*, 465
Lamium amplexicaule, pollen tubes of, 477
Laportea canadensis, 398
Lathyrus, pollen of, 472
Leguminosae, 66; food plants of, 329
Leontodon Taraxacum, 283
Leptosporangiatae, 313
Leucophyllum texanum, 194
Levine, Michael. Studies on plant cancers III. The nature of the soil as a determining factor in the health of the beet, *Beta vulgaris*, and its relation to the size and weight of the crown gall produced by inoculation with *Bacterium tumefaciens*, 507
Liliaceae, food plants of, 329
Lilium pardalinum, pollen of, 472; *rubrum*, pollen of, 483
Lily, stylar canal of, 475; pollen of, 477
Liquidambar styraciflua, 194
Liriodendron, Aspergillus cultures on bark of, 113
Loganiaceae, 331
Lupine, 27; pollen of, 472
Lychnis, pollen of, 472
Lycopods, 314
Lygodium, 313; *articulatum*, 312
Lyon, C. J. Comparative studies on respiration XVIII. Respiration and antagonism in Elodea, 458
Lythrum salicaria, pollen of, 479

MacDougal, D. T. Water deficit and the action of vitamines, amino-compounds, and salts on hydration, 296
Maclura pomifera, floral anatomy of, 394
Macroglossum, sporophyte of, 308
Magnolia, 194; pollen of, 483
Mains, E. B. Unusual rusts on Nyssa and Urticastrum, 442
Maize endosperm, genetic evidence of aberrant chromosome behavior in, 411
Malachra capitata, in Texas, 195
Mallow, pollen tubes of, 474
Malva parviflora, 195; *sylvestris*, 203
Malvaceae, 331; food plants of, 329; in southern and western Texas, distribution of, 192
Malvales, floral structure of, 405
Malvastrum americanum, 194; *coccineum*, 200; *elatum*, 200; *spicatum*, 195; *wrightii*, 195
Malvaviscus arboreus, 203; *drummondii*, 194
Mangel wurzel, crown gall of, 508
Maple pollen, 477; 483
Marattiales, anatomy of, 309
McDougall, W. B. Thick-walled root hairs of Gleditsia and related genera, 171
McNair, James B. A study of *Rhus diversiloba* with special reference to its toxicity, 127; the morphology and anatomy of *Rhus diversiloba*, 179; the transmission of Rhus poison from plant to person, 238
Mechanism of root pressure and its relation to sap flow, 369
Melampsora, 443
Melampsoridium Betulae, 444
Melampsoropsis, 447, 448
Melica ciliata, 44
Mesquite, in Texas, 194, 198
Mexican palm, in Texas, 194
Mignonette, stylar canal of, 475
Millet, the Linnaean concept of pearl, 41
Mimosa, 198
Modiola caroliniana, 194
Monocarpy and pseudomonocarpy in the Cycadeoids, 218
Moraceae, food plants of, 330; floral anatomy of, 387
Morus alba, floral anatomy of, 394; *nigra*, 394
Mountain cedar, 198
Musaceae, 329, 331

Narcissus, pollen of, 471
Nematogonium aurantiacum, 122
Nitrogen, the fixation of free, by green plants, 1
Nolina, 199
Nyssa, 194; and Urticastrum, unusual rusts on, 442; *aquatica*, 442; *capitata*, 443

Oak, 198; pollen tubes of, 474
Oat plant, the relation of certain nutritive elements to the composition of, 256
Oats, Ligowa, 455; miscellaneous studies on the crown rust of, 452
Oenothera, 193; pollen tubes of, 474
Oleaceae, 329
Ophioglossum Moluccanum, 304; *pedunculosum*, 304; *pendulum*, 313; *reticulatum*, 305, 313
Opuntia, 194
Orchidaceae, 387
Osmotic concentration of the guard cells during the opening and closing of stomata, variations in the, 30
Osmotic pressure, suggestions with respect to the measurement of, 164
Otomycosis aspergillina, 125
Ottonia section of Pipers, 212
Overton, James Bertram. The mechanism of root pressure and its relation to sap flow, 369

Palm, Mexican, 194
Palmaceae, food plants of, 329
Pandanales, 219
Panicum, 193, 253; *alopecuroides*, 43; *americanum*, 41, 43; *cynosuroides*, 44; *dichotomum*, 48; *glaucum*, 41; *italicum*, 253; *lutescens*, 41; *miliaceum*, 46, 253; *spicatum*, 41, 47; *viride*, 43
Papaveraceae, 330
Papilionaceae, 405
Papilionoideae, 175
Parietales, 405
Parkinsonia aculeata, 194; *texana*, 197
Paton, Julia Bayles. Pollen and pollen enzymes, 471
Pavonia lasiopetala, 199
Peach curl, 318; yellows, 316
Pear blight, 316
Pecan, 198
Pelargonium, pollen of, 472
Penicillaria spicata, 41
Pennisetum alopecuroides, 41; *americanum*, 41, 43; *compressum*, 43; *glaucum*, 41;

polystachyum, 44; *sieberianum*, 43; *spicatum*, 43; *typhoideum*, 41
Pennypacker, John Y. (See J. Arthur Harris, 63, 339, 375, 425)
Permeability, the effect upon, of (I) the same substance as cation and anion, (II) changing the valency of the same ion, 464; of plant cells, quantitative study of the effect of anions on the, 366; of polyvalent cations in combination with polyvalent anions, effect upon, 382
Perotis latifolia, 43
Pestalozzia, on Sapodilla, 523
Petunia, pollen of, 479
Phakopsora, 447
Phaseolus multiflorus, experiments with, 291; *vulgaris*, correlations between anatomical characters in the seedling of, 339; interrelationship of the number of the two types of vascular bundles in the transition zone of the axis of, 425; normal (dimerous) seedling of, 66; trimerous seedling of, 69; vascular anatomy of dimerous and trimerous seedlings of, 63; vascular anatomy of hemitrimerous seedlings of, 375
Phlox, pollen of, 472
Phyllosticta, 320
Physopella, 447
Pilobolus, 371
Pinaceae, 331
Pine, 198; pollen of, 471, 483; blister rust, 316
Piñon, 198
Pinus Coulteri, 223; *edulis*, 199; *attenuata*, 223; *palustris*, 194; *taeda*, 194
Piper **abalienatum**, 213; **albicaule**, 213; **brachypus**, 213; **Diguetianum**, 214; *discophorum*, 213; *Jaborandi*, 212; **Mas**, 215; *Muelleri*, 213; *Neesianum*, 213; *ovatum*, 213; **Rosei**, 215; *smilacifolium*, 213; **Tatei**, 213; **Thiemeanum**, 213; *yucatanense*, 213; *Zippelia*, 213
Plant pathology, the relation of, to human welfare, 315
Plantago lanceolata, pollen of, 479; *major*, 282
Platanus, Aspergillus cultures on bark of, 113; *occidentalis*, 437
Poa pratensis, 255
Poaceae, 328, 331
Poison ivy, 127, 130; oak, 127, 130, 132, 133; of *Rhus*, 137; vine, 127
Poison, Rhus, transmission of, from plant to person, 238
Pollen and pollen enzymes, 471
Polygonaceae, 328; food plants of, 329
Potato, 257; late blight fungus, 316; losses from disease, 315; wart, 316
Prepinus, 224
Primula obconica, 244
Prosopsis glandulosa, 194
Protococcus sp., 2, 18, 19, 20, 21, 22
Protosiphon botryoides, 2, 18, 19, 20, 21, 22
Pseudomonocarpy, monocarpy and, in the cycadeoids, 218
Puccinia, 236; *coronata*, 452; *Distichlidia*, 282; *glumarum*, 523; *graminis*, 453

Quantitative study of the effect of anions on the permeability of plant cells, 366
Quercus alba, leaves of, 433; *michauxii*, 194; *stellata*, 194; *virginiana*, 197

Raber, Oran L. A quantitative study of the effect of anions on the permeability of plant cells, 366; the effect upon permeability of polyvalent cations in combination with polyvalent anions, 382; the effect upon permeability of (I) the same substance as cation and anion, and (II) changing the valency of the same ion, 464
Ragweed pollen, 473
Ranales, floral anatomy of, 403
Ranunculaceae, 330
Respiration, comparative studies on, XVIII. Respiration and antagonism in Elodea, 458
Rhododendron, stylar canal of, 475
Rhus Cotinus, 184; *diversiloba*, 238; *diversiloba*, morphology and anatomy of, 179; *diversiloba*, study of, with special reference to its toxicity, 127; *lobata*, 128; *radicans*, 127, 238; *Toxicodendron*, 127, 180, 239; var. *radicans*, 183; *venenata*, 248; *vernicifera*, 137, 181, 244; *Vernix*, 137, 188, 239
Rhus poison, transmission of, from plant to person, 238
Rice smut, 319
Richardia, pollen of, 472
Robinia, 27; *Pseudo-Acacia*, 175
Root hairs, thick-walled, of Gleditsia and related genera, 171
Root pressure, mechanism of, and its relation to sap flow, 369

Roots, grain, note on the histology of, 207
Rosaceae, 330; food plants of, 329
Rosales, floral anatomy of, 403
Rubiaceae, 330
Rudbeckia, 193
Rutaceae, 219; food plants of, 329
Rye, pollen of, 480

Sabal mexicana, 194
Sabbatia, 193
Sagittaria, embryogeny of, 505
Salt proportions and concentration, effect of, on the growth of *Aspergillus niger*, 147
Salvia, pollen tubes of, 476
Sap flow, mechanism of root pressure and its relation to, 369
Sapodilla, inoculated with *Pestalozzia* sp., 523
Saprolegnia monilifera, 232; *torulosa*, 235
Saprolegniaceae, Isoachlya, a new genus of, 231
Sarracenia purpurea, pollen tubes of, 476
Saxifraga sarmentosa, 291
Scenedesmus sp., 2, 14, 15
Schizolobium giganteum, 220
Schroeteriaster, 447
Screw-bean, 199
Seedlings, trimerous and dimerous, 354
Senecio Doria, 31
Septoria, 320; *apii*, on celery, 523
Seradella, 27
Serratula arvensis, 283
Setaria glauca, 41; *lutescens*, 41
Siberian crab-apple, pollen of, 483
Siblings, correlation between bundle number in, 357
Sida angustifolia, 195; *ciliaris*, 195; *cuneifolia*, 195; *diffusa*, 195; *filiformis*, 195; *hastata*, 195; *longipes*, 200; *neomexicana*, 200; *rhombifolia*, 194; *spinosa*, 194; *tragiaefolia*, 200
Siderocarpus flexicaulis, 194
Silene, pollen of, 472
Sinnott, Edmund W. (See J. Arthur Harris, 63, 339, 375, 425)
Smuts, 318
Solanaceae, 331; food plants, 329; useful plants of, 330
Sorghum, 44; smut, 316
Spartina, 194
Spathelia simplex, 219
Spathyema foetida, fertilization of, 476
Specialization and fundamentals in botany, 275
Sphaeralcea cuspidata, 198; *hastulata*, 198; *incana*, 200; *lobata*, 196; *pedatifida*, 198; *pumila*, 204; *subhastata*, 199; *tenuipes*, 200
Sporobolus, 194
Steffensia, 212
Stelar theory, the eusporangiate ferns and the, 303
Sterigmatocystis albo-lutea, 122; *butyracea*, 122; *castanea*, 120; *pseudoflava*, 125
Stevens, F. L. The relation of plant pathology to human welfare, 315
Stichococcus sp., 2, 14, 15
Stomata, variations in the osmotic concentration of the guard cells during the opening and closing of, 30
Strombocarpa pubescens, 199
Stropholieion Californicum, 144
Systematic botany, the type concept in, 251

Taraxacum, 283; *densleonis*, 283
Taxaceae, pollen tubes of, 474
Taxodium distichum, 194; *mucronatum*, 229
Taylor, William Randolph. The embryogeny of *Cyrtanthus parviflorus* Baker, 502
Temperature, influence of, on the relations between nutrient salt proportions and the early growth of wheat, 59
Thom, Charles, and Margaret B. Church. *Aspergillus flavus*, *A. oryzae*, and associated species, 103
Tiliaceae, 331
Tilletia horrida, 319
Timothy pollen, 473
Tomatoes, losses from disease, 315
Toxicity of *Rhus diversiloba*, 127
Toxicodendron triphyllum glabrum, 127
Trelease, William. North American Pipers of the section Ottonia, 212
Trisetum, 255
Triticum vulgare, 207
Tropaeolum, pollen of, 472
Tsuga, Aspergillus cultures on bark of, 113
Tulipa, pollen of, 472
Type concept in systematic botany, the, 251
Typha elephantina, pollen of, 473

Ulmaceae, floral anatomy of, 387
Ulmus, 194; *americana*, 388; *campestris*, 388; *fulva*, 388; *racemosa*, 388; *scabra*, 388
Umbelliferae, 330; food plants of, 329
Umbrella palm, 219
Uniola, 255
Uredo Nyssae, 442

Uredopeltis, 447
Uromyces, 236
Urtica gracilis, 397
Urticaceae, 331, 387
Urticales, floral anatomy of the, 386
Urticastrum, unusual rusts on Nyssa and, 442; *divaricatum*, 444

Vacciniaceae, 330
Vachellia Farnesiana, 194
Variations in the osmotic concentration of the guard cells during the opening and closing of stomata, 30
Vascular anatomy of dimerous and trimerous seedlings of *Phaseolus vulgaris*, 63; of hemitrimerous seedlings of *Phaseolus vulgaris*, 375
Vein-islets in leaves of certain plants as an age determinant, area of, 433
Venturia, 319
Verbascum Lychnitis, 31
Veronica incana, 31
Violet, pollen of, 471; stylar canal of, 475
Vitamines, amino-compounds, and salts on hydration, water deficit and the action of, 296
Vitis, pollen tubes of, 474; *vulpina*, leaves of, 433

Wallflower, pollen of, 471
Wann, Frank B. The fixation of free nitrogen by green plants, 1
Water deficit and the action of vitamines, amino-compounds, and salts on hydration, 296
Wheat, influence of temperature on the relations between nutrient salt proportions and the early growth of, 59
Wieland, G. R. Monocarpy and pseudomonocarpy in the cycadeoids, 218
Wiggans, R. G. Variations in the osmotic concentration of the guard cells during the opening and closing of stomata, 30
Willow, 199
Wissadula holosericea, 200; *lozani*, 196; *periplocifolia*, 196

Yucca, 198; *filamentosa*, 218

Zamia, pollen of, 472; *Skinneri*, pollen of, 472
Zea Mays, 207; Aspergillus on, 118, 121; pollen tubes of, 474
Zebrina pendula, 33, 34, 164
Zostera, pollen of, 473

THE NEW ERA PRINTING COMPANY

LANCASTER, PA.

Is prepared to execute in first-class and satisfactory manner all kinds of Printing, Electrotyping and Binding. Particular attention given to the work of Schools, Colleges, Universities and Public Institutions

Books, Periodicals

Technical and Scientific Publications

Monographs, Theses, Catalogues

Announcements, Reports, etc.

All Kinds of Commercial Work

Publishers will find our product ranking with the best in workmanship and material, at satisfactory prices. Correspondence solicited. Estimates furnished.

THE NEW ERA PRINTING COMPANY

NATIONAL PHOTO-ENGRAVING CO.

46-48 NEW CHAMBERS STREET

NEW YORK CITY

Telephone; BEEKMAN 3179

PRODUCERS OF HIGH-CLASS ENGRAVINGS
HALF - TONES, LINE PLATES, COLOR WORK, Etc.

Plate-makers to

AMERICAN
JOURNAL OF BOTANY

RARE AND STANDARD BOOKS

FOR SALE AT 1914 PRICES

By A. J. Grout, New Dorp, Richmond Co., New York City

Mosses with Hand-lens and Microscope, bound in cloth, $7.00. 8 x 11 inches, 416 pages, 88 full-page plates and 265 figures in the text.

Mosses with a Hand-lens (224 pp., 8vo, copiously illustrated) is a smaller book describing 250 mosses and 50 hepatics that can be identified by means of the Hand-lens alone. Specially adapted for beginners. $1.75.

The Moss Flora of New York City and Vicinity, Grout. 121 pages, 12 full page plates, lists 380 species and varieties. Full keys and descriptions of families and genera. $1.25.

Bryologist, 22 vols., complete to Dec. 1919; vols. 1–10, bound in buckram with 10-yr. index, $25. American Botanist, vols. 1–18 complete, $10. Bull. Torrey Bot. Club, vols. 1–47 (lacking 27 nos. in vols. 1–10), 14 vols. well bound, $125. Am. Naturalist, vols. 1, 27, 29, 30, 31, 32, 34, complete, 33—lacks nos. 2 and 7, odd nos. of vols. 6, 21, 22, 24, 28, $15. Bot. Gazette, vols. 1–54 (1–12 lack 68 nos.) (23–30 well bound, also 35 and 36), $150. Bull. N. Y. Bot. Gardens, vols. 1–17 complete, $15. Fern Bull., vols. 8–20 complete, $13. Also odd nos. of 1–7, Nature Study Review, vols. 1–16 complete, $15. Journal of Mycology, vols. 11 and 12 complete, odd nos. of vols. 5, 9 and 10, lot, $4. Ohio Mycological Bulletin, 5 vols., nos. 1–84 (lacks 9 and 12), $5. Ohio Naturalist, vols. 1, 1–8; vols. 2, 1–6, $1. Rhodora, vols. 15–22 complete, $7. Torreya, 20 vols., complete to date, $19. Plant World, vols. 8 and 9 complete, vol. 1 (lacks nos. 8, 10, 12), $4.

Odd nos. of many vols. of most of the above for exchange or for sale to fill out sets. Will exchange or purchase Am. Botanical publications. See also Jan. to Nov. Advs.

Clarke Memoir Paper

A Permanent Record Paper with Half Tone
and Color Printing Qualities

CLARKE & COMPANY

711 Brunswick Building 225 Fifth Avenue
NEW YORK

Professional Advancement Service

The Professional Advancement Service seeks to aid men and women in botany and related subjects in securing such positions as they may desire, and to offer to heads of departments, or directors of laboratories, experiment stations, or other institutions an opportunity of making known the vacancies they seek to fill.

The method of the service consists in publishing in the American Journal of Botany a brief notice stating the training and experience of the applicant or the requirements of the vacancy. Each person using the Service is designated by number and his identity may be learned on application to the Business Manager of the American Journal of Botany. A list of eligibles and open positions is kept, and inquiries are referred to the advertisers who are mentioned or seem best suited to the requirements stated. The Professional Advancement Service may thus have the opportunity to mention a man for a desirable position or a promotion a number of times during a period of several months. The charge for the service is $2.00 for each case for one year or less. The notice regarding qualifications offered or desired will be published at frequent intervals, but not more than ten times.

Address: The Business Manager, American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y.

No. 37. Woman, B.S., Ph.D. Head of Biology Department in a state normal college, head of department of botany in college. Nine years' experience; experience also in bacteriology. Desires position combining research with teaching.

No. 38. A botanist and agronomist with four years' experience teaching college botany and four years' experience in agronomic work with the U. S. D. A. desires a position as Professor of Agricultural Botany. Has specialized in the practical application of botany to agriculture. B.S. and M.S. degrees and over 30 publications.

No. 39. Botanist with A.B. and A.M. degrees, expecting to receive his Ph.D. degree from a large eastern university in Sept., 1921, desires a teaching, or preferably a teaching and research position, in a college or university. Six years teaching experience; also experience in extension and field work in pathology in B. P. I., U. S. Dept. of Agriculture. Several publications.

No. 40. Ph.D. (eastern university), June, 1921, broad training in zoology, physiology, chemistry, and bacteriology, besides botanical sciences (morphology, pathology, mycology, cytology, physiology). Teaching experience in eastern universities, and in land-grant college. Recommendations of the best description as to abilities in teaching and research.

No. 41. Professor of Botany (Ph.D., 1909) with fourteen years teaching experience, nine in the present location, wishes a change of position. Special interest in Physiology and Pathology. Teaching with ample time for research, or full time research in fungous nutrition especially desired.

No. 42. Western state university needs young man as assistant or instructor; bachelor's degree required, with at least 20 hours botany and 10 hours chemistry. Applicant should speak English correctly and as a matter of habit. A modest knowledge of Latin is necessary. Initial salary $1,200 to $1,400 per annum. Work begins Jan. 1, 1922, or later. The man appointed will be expected to do some graduate work in botany or zoology.

No. 43. Botanist, possessing B.A. and M.A. degrees from a leading university, desires position in Eastern college, university, or collegiate institute. Is interested chiefly in Morphology, Systematic Botany, and Ecology. Has eleven years' experience in college teaching and has published several books and papers on botanical subjects.

WHELDON & WESLEY, Ltd.

THE SCIENTIFIC AND NATURAL HISTORY BOOKSELLERS,

Have in stock nearly all requirements in books, pamphlets, and periodicals relating to Chemistry, Agriculture, Gardening, Forestry, Botany (Systematic, Economic, and Geographical), Zoology, (including Ornithology, of which the stock is probably the largest in the world, Systematic and Economic Entomology, works on Mammalia, Reptilia, Fish and Fisheries, Mollusca, Invertebrata, etc.), Microscopy, Geology, Palaeontology, Mineralogy, Mining, Metallurgy, etc., both new and second-hand.

Catalogues of any subject, price 2d. each. Any book quoted on application, and those not in stock sought for without charge.

We beg to draw attention to the fact that we hold the largest stock in the country of

JOURNALS AND TRANSACTIONS OF SCIENTIFIC SOCIETIES,

Including Transactions and Proceedings, etc., of the Royal Society, Linnean Society and Zoological Society of London, Journals of the Chemical, Chemical Industry, Geological, Geographical, Royal Microscopical, Royal Agricultural, Horticultural, Astronomical, Physical, and other Societies, Proceedings of numerous local Natural History Societies, also Nature, Ibis, Zoologist, Curtis's Botanical Magazine, Journal of Botany, and numerous other Periodicals, British and Foreign.

Sets, runs, odd vols., and numbers quoted for on application.

Subscriptions taken for all Scientific and other Journals, which will be posted regularly on issue.

Libraries and parcels of Natural History and Scientific Books purchased.

38, GREAT QUEEN STREET, KINGSWAY, LONDON, W.C. 2.
Telephone: GERRARD 1412,
AND 28, ESSEX STREET, STRAND, W.C. 2.
(Telephone: CITY 2322).
BOOKBINDING UNDERTAKEN BY EXPERIENCED WORKMEN.

PHOTOMICROGRAPHS

HIGH GRADE RESULTS MADE FROM YOUR MATERIAL

BLACK AND WHITE OR DIRECT COLOR PHOTOS

ORDINARY OR POLARIZED LIGHT

LANTERN SLIDES OR PRINTS FURNISHED

PLAIN OR HAND-COLORED SLIDES OF BOTANICAL SUBJECTS FOR SALE

Ask for subjects desired

ARTHUR G. ELDREDGE

Box 83, Univ. Sta. Urbana, Illinois

BOTANICAL SOCIETY OF AMERICA

Reorganized 1906, uniting the Botanical Society of America (1893), the Society for Plant Morphology and Physiology (1896), and the American Mycological Society (1903).

OFFICERS FOR 1921

President: C. E. ALLEN
University of Wisconsin

Vice-President: H. C. COWLES
University of Chicago

Secretary: J. R. SCHRAMM
Cornell University, Ithaca, N. Y.

Treasurer: IRVING W. BAILEY
Bussey Institution, Harvard University

Councilors:

N. L. BRITTON
New York Botanical Garden

WILLIAM TRELEASE
University of Illinois

J. C. ARTHUR
Purdue University

Any one actively interested in botanical work is eligible for membership Candidates for membership are recommended by three members, not members of the Council, on blanks to be obtained from the Secretary.

The annual dues are $5.00, including subscription to the AMERICAN JOURNAL OF BOTANY. Candidates proposed for membership, and approved by the Council, may receive one copy of the JOURNAL at $4.00 per year pending their election at the winter meeting of the Society.

4
70

The Little Things That Count Big In Owning Your Own Greenhouse

B

Ever gone into a hat store, knowing exactly the kind and shaped hat you want; and then have some smart Alec of a salesman try to sell you the kind HE likes?

Makes a fellow madder than a wet hen, doesn't it?

Suppose, however, that same salesman had told you something about some of his hats that meant better service to you—more satisfaction for your money—you then sort of feel towards that salesman as you would an old friend.

Salesman number two is our sort when it comes to selling a greenhouse.

We do sell greenhouses, but we first sell satisfaction.

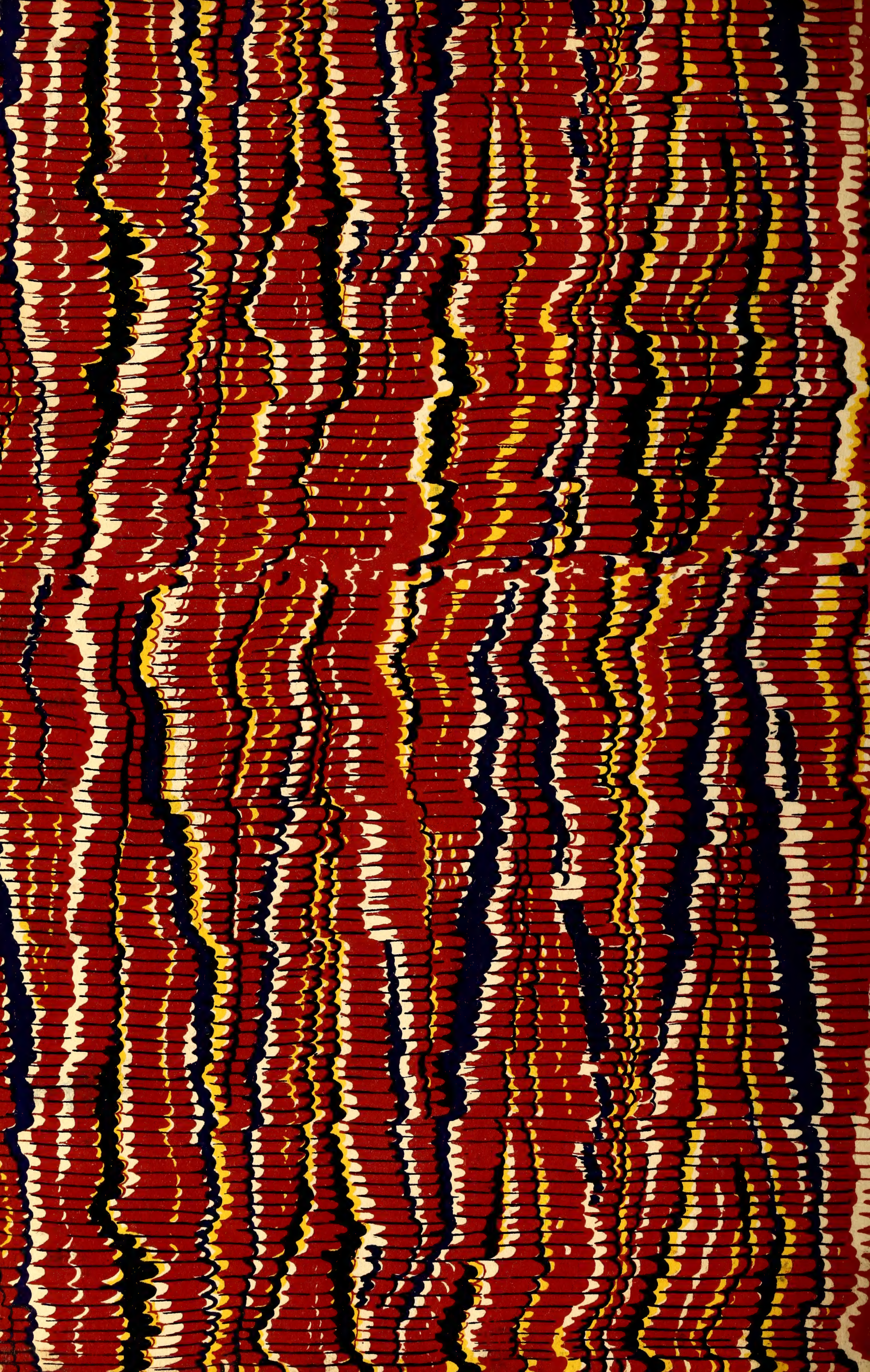
Catalog for the asking.

Hitchings and Company

Home Office and Factory: ELIZABETH, N. J.

NEW YORK
101 Park Ave.

BOSTON-9
294 Washington St.



SMITHSONIAN INSTITUTION LIBRARIES
3 9088 00691 0301